From the Department of Medicine, Solna, Karolinska Institutet,
Stockholm, Sweden

SUN EXPOSURE, PREVALENCE AND LOCALIZATION OF COMMON MELANOCYTIC NAEVI IN SWEDISH CHILDREN

Maria Karlsson

Karolinska Institutet

Stockholm 2015

Published by Karolinska Institutet.

Printed by Universitetsservice US-AB

ISBN 978-91-7549-810-2

Cover page: The author on the beach of Sudersand, Fårö 1968

To my family

Karolinska Institutet

Institutionen för Medicin, Solna, Karolinska Institutet

Sun exposure, prevalence and localization of common melanocytic naevi in Swedish children

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Welandersalen, ingång B2, plan 00, Hudkliniken, Karolinska Universitetssjukhuset, Solna

Fredagen den 6 februari 2015, kl. 09.00

av

Maria Karlsson

Huvudhandledare:

Docent Ylva Rodvall
Karolinska Institutet
Institutionen för folkhälsovetenskap

Bihandledare:

Professor Bernt Lindelöf
Karolinska Institutet
Institutionen för medicin, Solna

Professor Carl-Fredrik Wahlgren
Karolinska Institutet
Institutionen för medicin, Solna

Docent Kerstin Wiklund
Karolinska Institutet

Fakultetsopponent:

Professor Christian Ingvar
Lunds Universitet
Institutionen för kliniska vetenskaper, Lund

Betygsnämnd:

Professor Yvonne Brandberg
Karolinska Institutet
Institutionen för onkologi-patologi

Docent Harry Beitner
Karolinska Institutet
Institutionen för medicin, Solna

Docent Ingrid Synnerstad
Linköpings Universitet
Institutionen för klinisk och experimentell medicin

Stockholm 2015

ABSTRACT

Background

The rapidly rising incidence rates of cutaneous malignant melanoma call for more effective prevention strategies. Melanoma susceptibility is associated with having many common melanocytic naevi. Having fair skin, blonde/ash-blonde hair and grey/blue/green eye colour are also joint risk factors for melanoma and naevi. Naevi appear in early childhood and are inducible by sun exposure. As latency between sun exposure and subsequent melanoma development may stretch for decades, naevi prevalence in children has been suggested as a suitable surrogate marker to monitor population trends in sun exposure.

Aims

The aims of this thesis were to analyse the effects of sun exposure and sun protection on naevi prevalence in Swedish children. Further, to review changes in body-site distributions of naevi in response to different patterns of sun exposure and to compare childhood naevi distributions with melanoma localization in adults at different latitudes of residing. The aim was also to validate the feasibility of mobile teledermatology for the remote assessment of naevi prevalence compared with standard manual counting of naevi.

Methods

Naevi prevalence and body-site localizations were investigated in two consecutive population-based cross-sectional studies conducted among 7-year-old children in southern Sweden in years 2002 and 2007. Survey data regarding children's sun exposures were provided by parental questionnaires. For comparison between childhood naevi and melanoma distributions in northern and southern Sweden, the Swedish Cancer Registry was utilized. Mobile teledermatology was performed with an iPhone 4S camera of naevi on the back of 97 children aged 7-16 years and naevi were counted by standard manual procedure. Inter-method reliability (i.e. mobile teledermatology versus manual counting) and inter-rater reliability (between two independent dermatologists) were calculated with weighted kappa statistic.

Results

Significant improvements in parental sun protective measures for their children were reported in 2007 when compared with 2002. Correspondingly, the total mean number of naevi per child had become significantly lower in 2007. Analysing the body-site specific naevi densities demonstrated that these had become significantly lower solely on intermittently sun exposed body sites, such as the trunk and limbs. The reduction of naevi was largest among boys. Comparisons between melanoma and childhood naevi distributions in northern and southern Sweden demonstrated an almost two-fold higher incidence of melanoma and likewise a higher density of childhood naevi in southern Sweden. Gender profiles and body-site distributions of childhood naevi matched significantly with melanomas in young and middle-aged adults. In southern Sweden slightly more naevi and melanomas were located on the trunk; a body site associated with intermittent sun exposure. Validation of mobile teledermatology imaging for the remote counting of naevi proved substantial for both dermatologists counting naevi from digital images compared with the manual assessment.

Conclusions

In summary, the results of this thesis supported and extended the scientific basis for which naevi in children can be used as objective biomarkers of sun exposure. It also supported childhood naevi to be largely consistent with overall and subsite distributions of melanoma in relation to gender and latitude of residing. Mobile teledermatology proved valid for estimating naevi prevalence on the back and could provide a feasible methodology following trends in sun exposure in children. In a future perspective, assessment of childhood naevi may be implemented in population-based surveillance programs validating the effectiveness of public health campaigns aiming to reduce incidence of melanoma in Sweden.

LIST OF SCIENTIFIC PAPERS

I. **M. A. Karlsson**, C.F. Wahlgren, K. Wiklund, Y. Rodvall
Sun tanning habits and prevalence of common melanocytic naevi among 7-year-old children in Sweden: changes over a 5-year period.
Br J Dermatol. 2011 Apr (164(4);830-7

II. **M. A. Karlsson**, B. Lindelöf, C.F. Wahlgren, K. Wiklund, Y. Rodvall
Mobile teledermatology is a valid method to estimate prevalence of melanocytic naevi in children.
Acta Derm Venereol. 2014. Aug 20. doi: 10.2340/00015555-1950

III. **M. A. Karlsson**, Y. Rodvall, C.F. Wahlgren, B. Lindelöf, K. Wiklund
Changes in the body-site distribution of common melanocytic naevi among 7-year-old Swedish children between year 2002 and 2007.
Manuscript submitted

IV. **M. A. Karlsson**, Y. Rodvall, C.F. Wahlgren, K. Wiklund, B. Lindelöf
The anatomic distribution of cutaneous melanoma in adults and melanocytic naevi in children: a population-based study in northern and southern Sweden.
Manuscript submitted

CONTENTS

1 BACKGROUND 7

1.1 COMMON MELANOCYTIC NAEVI 7

1.1.1 Introduction 7
1.1.2 Epidemiology 7
1.1.3 The melanocyte 8
1.1.4 The life cycle of common melanocytic naevi 9
1.1.5 Dermatoscopic patterns of childhood naevi 10

1.2 CUTANEOUS MALIGNANT MELANOMA 10

1.2.1 Epidemiology 10
1.2.2 Melanoma in children 11
1.2.3 The divergent pathway of melanoma 11

1.3 FACTORS RELATED TO THE DEVELOPMENT OF NAEVI 11

1.3.1 Phenotypes 11
1.3.2 Genotypes 13
1.3.3 Ultraviolet radiation 14
1.3.4 The immune system 14

1.4 SUN EXPOSURE IN CHILDHOOD 15

1.4.1 The skin in childhood 15
1.4.2 Different patterns of sun exposure 15
1.4.3 Parental influence on childhood sun exposure 16
1.4.4 Sun travels 17

1.5 SUN PROTECTION 17

1.5.1 Innate mechanisms for sun protection 17
1.5.2 Sunscreens 17
1.5.3 Physical sun protection 18

1.6 BEHAVIOUR AND ATTITUDES TOWARDS SUN TANNING 19

1.6.1 Historical overview 19
1.6.2 Global campaigns and health initiatives 20
1.6.3 Current trends in sun tanning and sun protection 21

1.7 MONITORING OF SUN EXPOSURE 23

1.7.1 Questionnaire surveys 23
1.7.2 Dosimeters 23
1.7.3 Thymidine dimers 23
1.7.4 Manual counting of naevi 23
1.7.5 Digital imaging of naevi and teledermatology 24

2 AIMS 25

3 MATERIALS AND METHODS 26

4 STATISTICAL ANALYSES 31

5 ETHICS APPROVALS AND CONSIDERATIONS 33

6 RESULTS 34

7 DISCUSSION 39

8 CONCLUSIONS 43

9 IMPLICATIONS AND FUTURE PERSPECTIVES 44

10 SUMMARY IN SWEDISH/POPULÄRVETENSKAPLIG SAMMANFATTNING 45

11 ACKNOWLEDGEMENTS 46

12 REFERENCES 49

LIST OF ABBREVIATIONS

α-MSH	*α-melanocyte-stimulating hormone*
ACTH	*adrenocorticotropic hormone*
ALM	*Acral lentiginous melanoma*
ASP	*Agouti signaling protein*
BRAF	*v-raf murine sarcoma viral oncogene homolog B1*
CI	*Confidence interval*
CIE	*Commission Internationale de d'éclairage*
DNA	*Deoxyribonucleic acid*
ICD-10	*10th revision of International Classification of Diseases*
IARC	*International Agency for Research on Cancer*
LMM	*Lentigo maligna melanoma*
MC1R	*Melanocortin 1 receptor*
MED	*Minimal erythema dose*
Melanoma	*Cutaneous malignant melanoma*
Naevi	*Common melanocytic naevi*
NM	*Nodular melanoma*
NMSC	*Non-melanoma skin cancer*
NRAS	*Neuroblastoma RAS viral oncogene homolog*
SD	*Standard deviation*
SNP	*Single nucleotide polymorphisms*
SPF	*Sun Protection Factor*
SSM	*Superficial spreading melanoma*
T=T	*Cyclobutane thymidine dimers*
UPF	*Ultraviolet Protection Factor*
UV	*Ultraviolet*
UVA	*Ultraviolet A radiation (315-400 nanometre)*
UVB	*Ultraviolet B radiation (280-315 nanometre)*
UVC	*Ultraviolet C radiation(100-280 nanometre)*

1 BACKGROUND

1.1 COMMON MELANOCYTIC NAEVI

1.1.1 Introduction

Common melanocytic naevi (naevi) are benign, light brown to black lesions that start to appear on the skin during the first years of life. They are also referred to as acquired melanocytic naevi, moles or birth marks. Naevi are hamartomas, meaning a local overgrowth of one or more normal cell types, often in the shape of a nodule or plaque. Naevi that are derived from melanocytes are entitled melanocytic naevi or naevocellular naevi and they come in many shapes and colourations, e.g. congenital melanocytic naevi, blue naevi, halo naevi, Spitz naevi and naevus spilus. In this presentation, only common melanocytic naevi are considered and are hereafter referred to as naevi.

Naevi share embryologic and genetic predisposing factors with melanoma and are likewise inducible by solar exposure (1-3). Having high numbers of naevi is conferred as the greatest risk factor associated with malignant melanoma (4-6). Whether naevi should be considered as a proxy for melanoma risk or if they, in themselves pose a risk of becoming malignant in the pathway of melanoma is debated (7). As latency between sun exposure and melanoma development may last for decades, naevi prevalence in children has been suggested suitable as an objective surrogate biomarker for studying the effects of different types of sun exposure and sun protection (8).

The overall aim of this thesis was to investigate the influence of sun exposure on the prevalence and localization of naevi in Swedish children. This may give important knowledge on how to implement better targeted sun protection for children, ultimately for preventing a further rise in melanoma incidence in Sweden.

1.1.2 Epidemiology

The number of naevi differs largely between populations worldwide and is generally higher among populations with fair skin, blonde/ash-blonde hair and grey/blue/green eye colour (4, 9). The latitude of residing brings differences in the ambient ultraviolet (UV) radiation which has been proven to be of importance even among very young children (10). For example, in a study by Green et al. the naevi prevalence was almost 7-fold higher in fair-skinned children aged 8-9 years living in Brisbane, Australia compared with same-aged children in the West-Midlands of England (11). In a study by Harrison et al. two parallel birth cohorts of babies of Celtic ancestry born in either Townsville, Australia (19°S) or Glasgow, Scotland (55°N) were followed over a three-year period (12). Results demonstrated that the proportion of Australian children with at least one naevus increased rapidly in the first 2 years of life from 65.2% at 12 months and 100% at 24 months. Corresponding proportions for the Scottish cohort were 30.5% at 12 months, 61.7% at 24 months and 83.6% at 36 months.

In Sweden the number of naevi among adults living at different latitudes have been investigated by Karlsson et al. (13), Rosdahl et al. (14) and Augustsson et al. (15). The results have demonstrated fewer numbers of naevi in Swedish inhabitants living close to the Arctic Circle (median number of 15 naevi per individual) as opposed to the populations in Linköping (median number of 23 naevi) and Gothenburg (median number of 53 naevi).

In 2001/2002 a cross-sectional, population-based survey was initiated by Rodvall et al to investigate the impact of parental sun protective regimens, travelling and sun burns on

number of naevi in children residing at different latitudes in Sweden (16). The study comprised the municipalities of Kiruna (67.8°N) and Piteå (65.3°N) in northern Sweden and Falkenberg (57.0°N) and Ljungby (56.9°N) in southern Sweden. Results demonstrated that the number of naevi were significantly fewer among children residing in Kiruna (5.6 (95% CI, 4.8-6.5)) and Piteå (6.2 (95% CI, 5.3-7.2)) compared with Ljungby (9.5 (95% CI, 8.2-11.0)) and Falkenberg (10.4 (95% CI, 8.9-12.0)). Within same level of latitude, children residing in a coastal location had higher number of naevi. The results clearly indicated that residing at latitudes with higher ambient UV levels, but also coastal living, was of major importance for the development of naevi in Swedish children.

These findings paralleled with the incidence of melanoma being much higher in southern Sweden and were well in line with the hypothesis that early UV exposure primes melanocytes for naevus formation as well as to a future potential malignant development. The results from this first study were the starting point for designing the four studies included in this thesis.

A brief summary of the basics of naevus development and the embryological, genetic and epidemiological associations with melanoma is described below.

1.1.3 The melanocyte

The melanocyte is a spindle-like, pigment-producing cell in the skin. It is derived from ectodermal tissue formed in the earliest stages of embryogenesis and shares a common ancestry with the nervous system (17). Melanocytes are present in the leptomeninges, the iris of the eyes, in hair follicles and throughout the basal layer of the squamous cell epithelia. The palms and hands hold an exception by the activity of the melanocytes being suppressed by factors produced by fibroblasts in the dermis (18).

The melanocyte is located in the basal layer of the epidermis in the skin and creates a basal pigmentary unit supporting 36-45 adjacent keratinocytes with dark-pigmented granules containing melanin. The melanin granules are stored in small carrier units, melanosomes, which are transported into the keratinocytes by the dendritic arms of the melanocyte (19). Here, the melanin functions as a physical and chemical barrier protecting the keratinocyte cell nuclei from DNA damage caused by UV radiation. In the human skin approximately every tenth cell in the basal layer is a melanocyte. The number of melanocytes is approximately the same irrespective of skin colour but in dark-skinned individuals the melanocytes are more efficient and contain larger melanosomes. There are approximately 2000 million melanocytes in the human skin, same in male and female and between ethnical groups (20). However, inter-individual variation is high and there are differences between body-sites. Highest melanocyte densities are found on the face (2012 melanocytes/mm^2) and groin/perineum (2380/mm^2) and lowest on the trunk (800-930/mm^2).

The substrate for melanin synthesis is the amino acid tyrosine. Tyrosine is processed by a series of oxidative steps by the enzyme tyrosinase (regulated by the *TYR* gene) to form either of the two forms of melanin existing in humans: eumelanin or pheomelanin. Eumelanin absorbs the whole spectrum of visible light and displays a black colouring. Pheomelanin absorbs all but the red wavelengths and gives a red-tinted reflection well recognized in red-haired individuals. A person's density of melanin and the proportions of eumelanin and pheomelanin give the full spectrum of hair, eye and skin colour characteristics. All forms of albinism are caused by dysfunction of *TYR* activity which leads to impaired pigmentation of the skin, hair and eyes (21).

Melanin production is regulated primarily by the melanocortin 1 receptor (MC1R). MC1R function is controlled by mainly by two agonists; α-melanocyte-stimulating hormone (α-

MSH) and adrenocorticotropic hormone (ACTH) and by an antagonist; Agouti signalling protein (ASP). Activation of the MC1R by an agonist stimulates the synthesis of eumelanin, whereas ASP can reverse those effects and elicit the production of pheomelanin (22). α-MSH as a slow-release subcutaneous injection has been used in experimental studies for the purpose of inducing skin pigmentation with the objective to prevent sun-related skin cancers. The use of α-MSH is controversial and it is sold as an internet drug under the epithet "Barbie-drug", promising a sun-tanned skin, increased libido and weight loss (23). Side effects such as nausea, darkening of naevi and development of eruptive naevi have been reported (24).

1.1.4 The life cycle of common melanocytic naevi

Melanocytic naevi are derived from melanocytes that have undergone a series of clonal changes to become naevus cells, also entitled naevocytes. The naevocytes lie in nests and have lost their ability to export melanin to adjacent keratinocytes. If naevi in themselves have a function or a purpose is not known but their presence in the skin can be seen among human populations all over the world. They are also well represented in other mammalian species, especially dogs and horses (25).

The formation of new naevi (naevogenesis) is a highly dynamic process that takes place throughout life but is most active during childhood, adolescence and young adulthood (5, 26, 27). In adults, the development of new naevi occur at a much slower rate and is paralleled by involution that in time gives a net reduction in the total number of naevi. A maximum naevi count is generally considered to occur around the age of 40 years, but follows an individual trait and is influenced e.g. by history of sun exposure, skin photo type and hereditary factors (28). With increasing age, a gradual reduction or fading of naevi occur. The process is slow and by most left unnoticed, likely because other pigmented lesions such as lentigo solaris and seborrheic keratosis increase in numbers.

Naevi are believed to undergo 3 phases in their natural history: inception to growth (birth), senescence (resting phase), and involution (death). Several theories regarding the life cycle of naevi have been proposed. The most predominant is the "top-down" theory saying that naevus precursor cells originate in the basal layer of the skin. They start forming nests in the basal layer and are then entitled *junctional naevi*. With time some of the naevus nests "drop down" in the dermis forming a *compound naevus*. If the naevus further matures, all naevus nests leave the basal layer and become stationary in the dermis forming a light brown or pale papule, an *intradermal naevus*.

In recent years, a theory of a dual pathway of naevogenesis has evolved saying that during embryogenesis small clones of precursor melanocytes migrate in either of two pathways (29). The *ventral pathway* evolves in conjunction to nerve sheets and gives rise to predominantly compound or intradermal naevi. Naevi that emanate from this pathway have been postulated to be influenced mainly by endogenous factors. The *superficial-dorsolateral pathway* comprises precursor melanocytes that are deported in the junctional zone of the dermis-epidermis, mucosa and hair follicles. It is hypothesized that these can be more readily activated by exogenous factors, such as UV radiation.

After having completed migration during embryogenesis, precursor naevocytes are left scattered in the skin (30). Under the influence of UV radiation and potentially other hitherto unknown triggers, a series of strategic hits are required to switch off the genetic down-regulation and the nevocytes start a clonal division, with time forming the different types of naevi. The volatility by which naevi in early adolescence appear and disappear is highest among those individuals with highest naevus counts (27). It is believed that several

senescence factors are operating in naevi and that lack of efficacy in any one is not sufficient to promote progression into malignant transformation (31).

1.1.5 Dermatoscopic patterns of childhood naevi

A dermatoscope is a hand-held optical device that provides 10 times magnification when held firmly against the skin. It is an invaluable diagnostic tool in the clinical practice of dermatologists and medical professionals diagnosing skin lesions and an aid to decisions regarding surgical interventions.

Analyses of the dermatoscopic patterns of naevi provided by longitudinal observational studies have strongly contributed to our knowledge on the formation of naevi (32, 33) and the findings have even suggested for a new classification (34). Four basic patterns characterize naevi in children: *globular, reticular, complex* (both globular and reticular) and *homogenous* (neither globular nor reticular) (35). The dominating dermatoscopic pattern of new naevi in children is globular. Globular naevi are hypothesized to originate from the ventral pathway of migrating melanocytes during embryogenesis. Globular naevi display a "congenital" phenotype and are postulated to be influenced mainly by hereditary factors. As children grow older they attain more of reticulate patterned naevi (28). These naevi display a dermatoscopic pattern corresponding to *junctional naevi* and do not convert to the globular pattern of a compound naevi with time. Reticular naevi are considered more dynamic and to a larger extent influenced by solar exposure.

The Study of Naevi in Children (SONIC) started 2004 among 5th graders in North America and has reviewed the basic dermatoscopic patterns in children and has found them to vary not only by age but also with body-site localization and skin photo type. For example, globular naevi are significantly more frequent and larger on the upper than the lower back (36). Reticular naevi are more prevalent in children with darker skin and the naevi have a smaller size in children with darker pigmentation. The researchers involved in the SONIC studies hypothesize that the development of naevi is modulated by host characteristics and that subsets of acquired naevi are biologically distinct, supporting divergent pathways of naevogenesis.

1.2 CUTANEOUS MALIGNANT MELANOMA

1.2.1 Epidemiology

Melanoma is overwhelmingly a disease of fair-skinned populations (9, 37) and the incidence has been rising dramatically over the last decades (38). The first to assess a relationship between sun exposure, expressed as latitude of residence, and melanoma mortality were Lancaster et al. in 1956 (39). These studies demonstrated significantly increased melanoma risk among Anglo-Celtic populations residing in tropical and subtropical parts of Australia, New Zeeland, South Africa, and United States compared with those residing within more temperate regions. Migration studies provided further evidence to support an increased risk of melanoma among north-Europeans immigrating to UV intense countries (40, 41). The risk proved to be directly related to the age of relocation and having moved before 10-years of age brought a comparable relative risk as the inborn, fair-skinned population (42, 43). The same correlations have not been ascertained for more darker-pigmented populations (44). Within Europe, the incidence of melanoma varies greatly between populations and the inhabitants of Scandinavia have among the highest incidence rates (45).

In later years there are reports of a decreasing trend in melanoma incidence among adolescents in Sweden (46) and in several other countries (47, 48). In a study in Queensland,

Australia there were indications as to a lowering of melanoma incidence in adults below 40 years of age on skin surfaces intermittently exposed to sun, such as shoulders, upper limbs and trunk (49). Another Australian study has demonstrated the incidence of thin melanoma in young people (15-24 years) in Queensland to be declining (50) and this trend has also been observed in the Swedish population (51).The results have been suggested to be attributed to improved surveillance techniques and earlier diagnosis alongside with a growing awareness of risky sun behaviour and skin cancer in the general population.

1.2.2 Melanoma in children

Colour or size changes in naevi in children may raise considerable concerns among parents; however melanoma in childhood is luckily exceedingly rare. In Sweden approximately 1 melanoma case per year appear in children below 14 years of age, 5-8 cases per year among 15-19 year-olds and 40-70 cases per year in 20-29 year-olds (52). Thus, there is clearly a breakpoint in adolescence when incidence rates start to rise rapidly (46, 53-55). Severe sunburns in childhood have been associated with melanoma in teenagers (56) and new genetic studies have linked UV overexposure also to childhood melanoma (57)

In adults, the clinical recognition of melanoma has been largely based on the macroscopic ABCDE melanoma detection criteria; (Asymmetry, Border irregularity, Colour variegation, Diameter >6 mm, Evolution). However in children, Cordoro et al has demonstrated that among 60 children aged 0-19 years diagnosed with melanoma, 40-60% did not present with conventional ABCDE criteria (58). Rather, amelanosis, bleeding, "bumps," uniform colour, variable diameter, and de novo development were common and this knowledge may facilitate earlier recognition of melanoma in children.

1.2.3 The divergent pathway of melanoma

The pattern of sun exposure (chronic or intermittent) on different body-site locations is known to generate different melanoma subtypes, generally referred to as the divergent pathway of melanoma as proposed by Whiteman and Green et al. (59). Lentigo maligna melanoma (LMM) associates with face/neck/ear localization, chronic sun exposure and actinic damage in the elderly. Superficial spreading melanomas (SSM) appear mainly in younger age groups, especially on intermittently sun exposed body sites in naevi-prone individuals. Nodular melanoma (NM) and acral lentiginous melanoma (ALM) are more ambiguous in their relation to sun exposure. The insight on how different patterns of sun exposure inflict both on naevi and melanoma subtypes, histogenetic characteristics and mutagenic status is a rapidly advancing field of research (60, 61).

1.3 FACTORS RELATED TO THE DEVELOPMENT OF NAEVI

1.3.1 Phenotypes

Skin, hair and eye pigmentation varies considerably among humans across the world (62). Native-born populations living close to the equator generally have dark complexions, effective in UV protection. The Out-of-Africa model proposes that *H. sapiens* approximately 200,000 years ago left east African territories and subsequently migrated through to Europe and Asia. The migration of populations over hundred thousand years of evolution has led to a gradual variation in human skin, eye and hair pigmentation. A number of hypotheses have been proposed for these colour adaptations: as a consequence of ambient UV exposure, to facilitate D-vitamin production in less UV intense environments, to enhance barrier function or be mediated by sexual preferences. (63, 64).

The human skin's propensity to burn and tan, i.e. the skin photo type, is generally evaluated using a standard method originally set by Thomas B. Fitzpatrick in 1975 (65). The skin photo types are divided in six classes (I-VI) based on the individual response to initial sun exposure corresponding to three minimal erythema doses (MEDs) (1 MED is equivalent to 15 to 30 minutes of unprotected noon exposure in northern (20° to 45°) latitudes) (Table 1).The individual's skin photo type is dependent on the basic, constitutional skin colour and is often, but not always, linked to certain hair and eye colours. Self-estimated skin UV sensitivity, according to Fitzpatrick's classification, has been shown to be a strong predictor of individual sun avoidance and sun protective behaviour (66). However, self-assessments of sun sensitivity may not always match with objective UV photo tests (67).

Table 1. Skin photo types according to Fitzpatrick's classification

Skin Photo Type	Basic skin colour	Characteristics[a]
I	Pale, very fair skin	Always burns, never tans
II	Fair skin	Usually burns, sometimes tan
III	Moderately fair skin	Sometimes burns, usually tan
IV	Light brown	Rarely burns, always tan
V	Medium brown	Never burns, dark tan
VI	Dark brown or black	Never burns, deep dark tan

[a] Classification is based on what patients say their responses are to initial sun exposure of three minimal erythema doses (MEDs) (1 MED is equivalent to 15 to 30 minutes of noon exposure in northern [20° to 45°] latitudes or 30 mJ/cm^2)

Having a fair skin photo type, being blue, grey or green eyed and having blond or ash blond hair associates with having many naevi and comes with an increased risk of melanoma (5, 9, 26). Interestingly, there is a discrepancy for individuals with red hair who, while having high risk of melanoma, generally have low numbers of naevi (68, 69). Facial freckling, when co-varying with red-hairiness, is inversely associated with having many naevi, while freckling in individuals with other hair colours is positively associated (70). Several loss-of-function variants of *MC1R* are highly correlated with red hair, poor tanning, freckling of the skin, and increased melanoma risk (71).

The naevus phenotype is of interest for clinicians making individual melanoma risk assessments in their patients. Dysplastic (or atypical) naevi are acquired naevi that are unusually large, show irregular pigmentation and which may, or may not, correlate with certain histopathological features (72). The term dysplastic or atypical naevi is widely used among clinicians and pathologists but with varying connotations. Large naevi follow an inherited trait and high counts correlate with greater overall melanoma risk (73). However, in the general population, sporadic dysplastic naevi are very common and the risk of progression to melanoma is low. Removal of clinically dysplastic nevi is not recommended as long as the lesion is not suspicious for melanoma (31). Dysplastic naevi are seldom a clinical issue in children but the body-site distribution of naevi may be informative and multiple or large naevi appearing in sun-shielded areas, such as the buttocks, may signal an inherited melanoma susceptibility (74).

Gender differences in naevi distributions between men and women are seen in adults (75). Males generally have more naevi situated on the face and trunk while females have more naevi on the extremities, especially the legs. This has been suggested to relate to different sun tanning patterns and clothing habits between the sexes. However, most naevi studies in children have also demonstrated the same gender profiles (76, 77).The most compelling evidence supporting that biological factors influence naevi distributions are studies performed among white Canadian Hutterite children (78). Throughout their childhood both boys and girls wear traditional costumes that provide near maximum sun protection. These children develop fewer total number of naevi (mean of 2.3) compared with non-Hutterite Canadian children (mean of 8.2) but still present with the typical gender patterns. The authors hypothesize that a differential regional tendency for melanocytes to proliferate exists in males and females.

1.3.2 Genotypes

The development of naevi is under strong genetic control interacting with environmental sun exposure (79, 80) . This has been demonstrated in studies of monozygotic and dizygotic twin pairs in Australia by Zhu et al (81) and in the UK by Wachsmuth et al (82). Being raised in Australia yielded consistently higher naevus counts compared with the UK population, but the same strong correlation in-between the monozygotic twins (r=0.94) and the dizygotic twins (r=0.60-0.64) was found (83). Twin studies have also demonstrated that naevi counts seem to be less influenced by genetic factors in younger age groups (<45 years) with a concordance estimated to 36% and the remaining variance being attributed to environmental factors. In adults > 45 years, up to 84% of naevi counts were estimated to be related to genetic factors (80).

The complex process of melanomagenesis is a continuously emerging field of research. The most common germ-line mutation that predispose to familiar melanoma with high penetrance is within *CDKN2A* (84, 85). *CDKN2A* is a tumour suppressor gene and mutations are considered of high-risk not only for melanoma but also for other forms of cancer (86). *CDKN2A* and other gene variants, e.g. *PLA2G6* and *TERT-CLPTM1L*, also predispose for multiple naevi (87). There are several pigment-determining genes, e.g. *TYR, OCA2, ASIP, MC1R, SLC24A5* and *MTAP*, whose activities provide the wide array of clinical pigmentary phenotypes known in humans (88). Small alterations, single nucleotide polymorphisms (SNPs) in one or several of these genes are common and vary within and between ethnic populations. Some of these mutations have been linked to melanoma susceptibility but are considered to be of a comparably lower risk (79, 89). Although the relative risk of familial melanoma is higher in young adulthood than in elderly, familial melanomas are not limited to young ages (90, 91).

Somatic mutations also relate to melanoma and naevi proneness. The first finding of a melanoma oncogene was *NRAS* in 1984 (92) wherein mutations are present in approximately 15% of melanomas. *NRAS* mutations are also detected in congenital melanocytic naevi (93). *BRAF* mutations in melanoma cell lines were first described by Davies et al in 2002 (94). Melanomas with *BRAF* mutations are linked to clinical characteristics such as younger age, SSM histogenetic subtype and localization on intermittently sun exposed sites (95-97). Further, *BRAF* mutations coincide with naevi-proneness and early-life sun exposure (98). However, *BRAF* mutations are not sufficient to precipitate melanoma and are present in approximately 80% of benign aquired melanocytic naevi (99, 100).

The finding of BRAF mutations in melanoma has opened a new field of therapeutic possibilities. However, BRAF inhibitors may activate other cellular pathways which may

cause multiple squamous cell carcinomas to arise as well as new wild-type BRAF melanomas (101). BRAF- positive naevi may involute with BRAF inhibitor treatment, while others grow and darken or multiple eruptive naevi may develop (102). This illustrates the complexity in melanocyte regulation involved in naevogenesis and melanomagenesis.

1.3.3 Ultraviolet radiation

UV radiation is one of the most well studied carcinogenic mediators and is also related to photo aging, immunosuppression and photosensitivity (103). It is strongly mutagenic by acting both directly and indirectly on the deoxyribonucleic acid (DNA) structure in the cell nucleus.

The UV spectrum ranges between 100 nm and 400 nm categorised into UVA (315-400 nm), UVB (280-315 nm), and UVC (100-280 nm). All UVC and most of the UVB is blocked by the stratospheric ozone layer. A fraction of UVB and all UVA reaches the surface of the Earth.

UVB is largely absorbed by the epidermis but to some extent penetrates to the papillary dermis (104). It induces high energy free radicals which break the chemical bounds in the nucleic acids in the DNA backbone and form different cyclobutane pyrimidine dimers (CPD). Most well-studied among the CPDs are the thymidine residuals that cross-bind to form thymidine-thymidine (T=T) dimers. T=T dimers are mainly induced by UVB with some contribution from UVA. (T=T) dimers risk altering the promotion and/or repression of the DNA transcription if occurring in the strategic oncogene-regions that steer cell proliferation. The DNA nucleotide excision repair system is constantly scanning the DNA strand for any missenses, cleaving off the (T=T) dimers as it passes. The detrimental effect of having a low-functioning DNA damage repair system can be seen in patients suffering from xeroderma pigmentosa, having an exceptionally high risk of developing skin cancers in the presence of UV (105).

UVA make up for 95% of the global UV radiation and causes DNA damage indirectly by generating reactive oxygen species. It penetrates deeper in the skin than UVB and reaches the papillary and upper reticular dermis (104). The role of UVB and/or UVA radiation and linkage to melanomagenesis is debated and associations are complex (106, 107). It has been proven that the body's repair and removal of damaged DNA was less effective when the damage was caused by UVA rather than by UVB (108). There is also evidence for an UV radiation-independent melanoma pathway emanating in individuals with red hair and fair skin photo types (109).

Artificial UV radiation, i.e. sun beds, for the purpose of sun tanning has been readily available in northern Europe and the United States since the 1980s (110). These lamps mainly produce UVA and sun bed use has been associated with subsequent risk of melanoma (111-113). Meta-analysis has demonstrated that the risk increases with number of sunbed sessions and with initial usage at a young age (<35 years) (114).

1.3.4 The immune system

Melanin production is influenced by multiple paracrine and autocrine factors excreted by keratinocytes, fibroblasts and immune cells homing in the skin. Skin inflammation has been shown to induce melanin production, not least by the inflammatory response induced by sunburns. However, pro-inflammatory cytokines and interleukins can also act in an inhibitory mode on melanogenesis (115). Clinical studies have shown a negative association between

numbers of common naevi in patients with classical inflammatory dermatoses such as atopic eczema (116) and psoriasis (117). The regulatory impact of the inflammatory system on melanocyte and nevocyte homeostasis can also be exemplified by multiple eruptive naevi that can arise after treatment with high-dose immunosuppressive drugs, cytostatic therapies or biologicals (118-120). Naevi often show infiltrates of lymphocytes and can eventually involute if the inflammation is pronounced, often clinically visible as a surrounding depigmented halo. These halo naevi are common events in children and adolescents. Immune-mediated mechanisms have been shown to play an important role in restraining the expansion of the premalignant melanocytes of naevi and early melanomas (31).

1.4 SUN EXPOSURE IN CHILDHOOD

It is estimated that approximately one-third of life time cumulative sun exposure is experienced before 18 years of age (121). Historically there is evidence that boys spend more time in the sun compared with girls (122). Defining a standard terminology for personal sun exposure is complex as it depends on a series of variables such as time of the day spent outdoors, latitude, altitude, weather conditions, body orientation in relation to incoming sunlight, reflections from the surroundings and local atmospheric conditions (123-125). Furthermore, innate factors such as skin photo type and the exhibiting of sun protective actions (e.g. clothing, sunscreens) are key determinants.

1.4.1 The skin in childhood

Although sun exposure over the whole life span inflicts on risk of skin cancer, early life is recognized as an especially susceptible time period for imprinting genetic changes involved in naevogenesis and melanomagenesis (126). This has also been given further substantial support in recent publications using animal models of baby mice and opossums (127, 128). A comprehensive explanation to the sensibility of child's skin is however not shown. The neonatal skin has all the anatomic structures developed at birth but has impairments regarding barrier functions and thermoregulation compared with adults. The capacity of melanocytes to shield from sun develops during the first months of life (129). Neonatal skin has within 6-8 weeks the same epidermal thickness as in adults but the horny layer, that thickens in response to physical stress may vary depending on age and body site. One theory on why childhood is a critical period has been linked to findings of melanocyte stem cells located in the hair follicle. Children have predominantly vellus hair which are located in upper dermis, thus being more vulnerable to UV radiation in comparison with terminal hair in adults that are located deeper in the skin (130).

1.4.2 Different patterns of sun exposure

Three major types of sun exposure on the body surface are generally referred to in the scientific literature: *chronic (continuous*), *intermittent* and *rare*. Generally the face, neck (in males) and dorsal aspect of the hands are considered chronically sun exposed. Body sites routinely covered with clothes but alternating with periods of uncovering, for example when at the beach swimming or sun bathing, are considered intermittently sun exposed. These areas are typically represented by the trunk and proximal extremities. Rarely sun exposed areas are considered the buttocks, genital area and, if hair is present, also the scalp.

Non-melanoma skin cancers (NMSC) predominantly arise on chronically sun exposed body sites as an effect of cumulative actinic sun damage. However, both naevi and SSM and NM melanoma subtypes have predilection sites on the trunk and limbs, being mainly intermittently sun exposed (131). Naevi and melanoma scarcely appear on strictly sun shielded body sites and are uncommon on the palms, soles, under the nail plate or on mucous

membranes. Occupational studies comparing indoor and outdoor workers have, somewhat surprisingly, demonstrated that indoor workers have an increased overall melanoma risk while outdoor workers have a lower than expected risk (132, 133). Beral and Robinson et al have shown that outdoor workers exhibit melanoma more commonly on the face, neck and ears while indoor workers have higher melanoma incidence on trunk and limbs (134).

The concept of chronic, intermittent or rare sun exposure is a rather crude measure when applied to larger body segments. For example, limbs are by most researchers considered intermittently sun exposed but the lower arms and legs can also be assigned as chronically exposed depending on the dress codes and clothing habits implicated by the climate in different countries. The amount of UV reaching the skin is also highly dependent on position; standing, sitting or lying down or whether still or in motion (135).

Sunburns in children most often occur on the face, shoulders and the back and numbers of sunburns have been linked to an increased number of naevi (136). Interestingly, most sunburns in children are reportedly experienced when performing habitual activities rather when on sun holidays which may be related to parental awareness being heightened during vacations (70). Although sunburns during childhood are associated with increase melanoma risk, sunburns throughout life are considered of importance (137, 138).

1.4.3 Parental influence on childhood sun exposure

By sharing everyday life, parents are likely to serve as role models for their children regarding attitudes to sun exposure, sunbathing and use of sun protective measures. These relationships have been issued, not least by Scandinavian researchers. In a study among Swedish parents with toddlers (1- 1,5-year-olds) it was demonstrated that if the parents attitudes towards tanning and sunbathing were positive, their children also spent more time in the sun and were more often sunburnt. (139). However, having the perception that sunbathing was harmful for the child increased the likelihood of the parent to use recommended ways of sun protection. A qualitative study conducted in 2009, seeking to analyse Swedish parents' perceptions of sun protection in young children, pointed out that use of sunscreen was widely implemented, but the parents reported significant uncertainty regarding its use and effectiveness. Staying inside during mid-day was the sun protective measure least parents were willing to adopt for their children (140)

In a recent Danish study the impact of parental sun practices were found to be restricted to the mother (141). The boys' use of sunscreen and girls' risk behaviour in the sun were related to their mothers' sun exposure and protection behaviour, whereas not to their fathers. The researchers thus suggested that future sun protection campaigns aimed at children should primarily involve the mothers.

The complex relationship between parental knowledge, attitude towards tanning and protective behaviour for young children has been investigated in a large population-based survey in Germany 2002 (142). Results demonstrated that even parents with a high level of knowledge about skin cancer risk factors did not protect their children adequately. That knowledge of risks of sun exposure is subordinate to a positive attitude towards a sun-tanned appearance has been demonstrated in adolescents and adults in studies by Bränström et al. (143, 144) . In Sweden, 99% of adults in Sweden are aware of the correlation between high levels of UV radiation and skin cancer (145). The results imply that future public health campaigns should not focus merely on the improvement of knowledge. Targeting tanned skin as a misguided ideal of beauty may be better suited to improve the current situation of skin cancer prevention.

1.4.4 Sun travels

The importance of sun travels for the rising melanoma trend is strongly implicated by cohort and case-control studies in adults (61). Travelling is directly linked to household disposable income and swedes are mobile (146). In 2012, 13 million flights to a holiday resort abroad were undertaken and there is still an upward trend in Sweden. Most popular destination is currently Spain, but in 2010, 443.000 swedes travelled to Thailand during the Swedish winter season which brings an extreme alteration in UV exposure. It may be hard to isolate the effect of sun travels from other types of recreational sun exposure experienced during weekends and domestic holidays. Sunburns are reportedly more often experienced at home compared with abroad which implies an underestimation of the risks of daily-life exposure, while better awareness when on sun holidays (147).

1.5 SUN PROTECTION

1.5.1 Innate mechanisms for sun protection

All living creatures have evolved innate mechanisms to prevent, decrease and repair UV mediated cell damage. Melanin functions mainly as a UV absorbent but also has antioxidant and free-radical scavenger properties (148). In addition to its role in pigmentation, MC1R is involved in the activation of DNA repair and other anti-photocarcinogenic activities (149).The thickening of the stratum corneum has an important role for reflecting and shielding of UVB and the degrading of filaggrin molecules within keratinocytes also act as natural sunscreens (150).

1.5.2 Sunscreens

Sunscreens have since more than 40 years been utilized for sun protection (151, 152). Sunscreens act either as filters which absorb within the UV spectrum or by scattering the incoming radiation. Protection from UVB is quantified by the sun protection factor (SPF). The SPF is based on the minimal erythema dose (MED) after applying 2 mg/cm^2 of sun cream on the skin. Proper application of an SPF 15 product, for example, allows a person to receive a 15-times higher cumulative UVR dose before exhibiting MED. The choice of sunscreen may depend on its pricing, cosmetic qualities, water-resistance and personal susceptibility to sunburn. However the protection against UVB by an SPF of 30 (97%) is not much less than that by an SPF of 50 (98%). While a significant primary preventive effect from regular sunscreen use has been demonstrated for actinic keratosis and NMSC (153) this was not until recently shown for melanoma (154).

The use of sunscreens, especially in young children, is not uncontroversial; some organic UV filters (PABA, cinnamates, benzophenones, and octocrylene) have been described to cause photo allergy. In animal studies, percutaneous absorption and endocrine disruption caused by nano-sized inorganic UV filters has been reported (155) and these products are currently withdrawn from the EU market. Modern large-molecular UVB-UVA broad spectrum sunscreens are regarded as having better benefit-risk ratio than former organic filters. Still, the effect is relying on sufficient and correct application and in animal models sunscreens have demonstrated less efficacy in preventing melanoma when compared with physical sun protection (128). In the last decade there has come compelling evidence that wavelengths present in natural sunlight beyond the UV spectrum also contributes to actinic damage and photo aging of human skin (156). The development of sunscreens that blocks parts of the visible spectrum is ongoing.

Why do frequent sunscreen users have more naevi and more melanoma?

Today it is well recognized that broad spectrum UVB and UVA protection with regular application in sufficient amounts are considered beneficial for prevention of skin cancers. However this was long a controversial issue due to repeated reports on higher naevi densities and higher melanoma risks in frequent sunscreen users. These associations are thought to be explained by several factors.

Firstly, using a sunscreen may introduce a **sense of false safety** which encourages towards staying longer in the sun. Sunscreens are readily wiped of and washed off by sweating or when swimming and the SPF label on the bottle seldom matches with the actual protection on the skin.

Secondly, spurious inferences may be drawn because most epidemiological studies are observational and the findings risk being inadequate for addressing causal relationships. This is because non-randomized studies are unable to distinguish the main determinants for sunscreen use from those with risk of skin cancer because they are the same; namely sun sensitivity with a susceptibility to sunburn. People who are prone to develop many naevi and skin cancer are therefore also more likely to be users of sunscreens. This is referred to as **indication bias** and can introduce a reverse causality between associations. Inherited factors, such as skin photo type, modify the likelihood of sunscreen use and act as **effect modifiers.**

References: (154, 157-159)

1.5.3 Physical sun protection

Physical sun protection is foremost advocated as the safest way to avoid carcinogenic effects of excessive sun exposure. It can be implemented by staying indoors or by seeking shade during mid-day or, if staying outdoors, by protecting the skin by clothing and wide-brimmed hats and the eyes by wearing sun glasses. In later years the use of especially designed sun protective clothing for children has emerged on the market. In an Australian study conducted in two cohorts of young children 1991 and 1999-2002, the reported use of sun protection had increased with popularity of swim-shirts and sunscreen, coinciding with less frequent sunburns on the trunk and with development of significantly fewer melanocytic naevi (160). The sun protective effect of the swim-shirts lies in the texture of the fabric giving an ultraviolet protection factor (UPF) of 25-50. The quality of the fabrics varies and by "wearing and tearing" the UPF can drastically decline (161).

The importance of providing outdoor environments offering natural shade for children has been investigated by Boldemann et al. These studies have shown that in preschools with many hills, trees and shrubbery the children receive lower levels of UV radiation (measured by dosimeters) but also higher levels of physical activity and, interestingly, they show less signs of behaviours coupled with inattention (162-164).

Sun advice for children
Children under one year of age should not be in direct sun light at all. Toddlers can be shaded with a parasol, sun tent or UV protection for their pram/pushchair, used in combination with the child wearing clothing and a sun hat.
Children regardless of age should be in the shade or indoors during mid-day when the sun is strongest (11 am- 3 pm)
The best sun protection for children is to use clothes, a hat and sun glasses. Sunscreen is best used as a complement for areas not covered by clothing.
Water resistant sunscreens, specially adapted for children are available and have a high sun protection factor (at least SPF 30).
The sun is usually stronger abroad than in Sweden. This makes protecting your child from the sun even more essential.

Recommendations from the Swedish Radiation Safety Authority

1.6 BEHAVIOUR AND ATTITUDES TOWARDS SUN TANNING

1.6.1 Historical overview

Before the industrial revolution in the 19th century, having pale skin was considered desirable and associated with wealth and nobility (165). When manual workers to a greater extent began to labour indoors, a gradual change in beauty ideal started. The discovery that rickets among children was related to depletion of sun light and dietary intake alerted the medical professionals to recommend sun exposure as a health bringing activity. Heliotherapy was also widely implemented in the treatment of systemic tuberculosis. Professor Niels Finsen was rewarded with the Nobel Prize in Physiology or Medicine in 1903 for his novel treatment with concentrated light radiation in patients with lupus vulgaris (166).

The fashion designer Coco Chanel proclaimed in Vogue 1929: "The 1929 girl must be tanned" and "A golden tan is the index of chic." This was the start of a trend were a life standard with excessive leisure time was manifested by a tanned, slim and elegant appearance and a clothing fashion that allowed for less covered dressing styles.

The children were in focus when campaigning for the benefits of early sun exposure. In the Ladies Home Journal in 1938 the following advice was given to mothers by a leading member of the medical profession (Bundesen H. Sunshine and health. Ladies Home J 1938 Aug; 55:48-5)

....When the baby is a month old, place him directly in the sunlight....Don't be afraid of the sun. Push the hood of the baby carriage down, take off the baby's bonnet, uncover his legs and feet. Place him on one side for a few minutes so that one cheek gets the sun's rays; then tum him on the other side....After the child is six months old, let him have his sunbath on his blankets spread out on the lawn.... But let us get out into the sunshine with the children as often as possible. The sunbath is just as important as the water bath.... Do this for your child's health now, this summer, and see how much healthier he will be in the year round.

By the 1960s and 1970s, sunbathing was an established part of the western culture coinciding with the start of sun travels and the use of artificial sun tanning parlours.

Birmingham Chest Clinic 1932 (from BBC News)

1.6.2 Global campaigns and health initiatives

Primary prevention strategies to diminish the risk of developing melanoma have been attempted worldwide (167). Australia, having the highest incidence of skin cancer in the world, has since the late 70's vigorously tried to counter the suntan fashion through educational campaigns. In 1980 Sid the Seagull recommended the population to "Slip on a shirt! Slop on a sunscreen! Slap on a hat!" and the campaign comprised free advertising in the mass media, community activities and teaching resources distributed to primary schools.

The "Sun Smart" campaign was launched in 1988 in Australia to encourage the use of more non-chemical sun-protection, i.e. focus less on use of sunscreens. Outdoor school activities were not scheduled at midday, shade trees were provided at school, and seeking shade at mid-day was encouraged.

In Sweden the first organized national campaign "Sola Sakta" was initiated in 1987. Free skin screening by a doctor was available in several beaches around Sweden during this summer. Media gave a spread of recommendations for sun protection and early detection of abnormal skin lesions. The campaign was followed by a peak in melanoma incidence in 1987-88 and in 1995-1996 there was a temporary levelling of the melanoma incidence which was perceived as an effect of the "Sola Sakta" campaign.

The Euromelanoma Day or "Melanoma Monday" is a screening campaign initiated by the European Academy of Dermatology and Venereology in 2000 (168). The main objective of Euromelanoma is to improve primary and secondary prevention of melanoma in the European population. Euromelanoma days have been implemented in Sweden since 2000 and have led to the discovery of a substantial numbers of melanomas (169, 170). The importance of early detection of melanoma is crucial because of the direct implication on prognosis and mortality if excising the melanoma when still thin (171).

In 2009 the International Agency for Research on Cancer (IARC) classified artificial UV emitting devises, such as commercial sun beds, as being carcinogenic and the use of sun beds below 18-years-of age is prohibited in many countries. An age limit to 18 years for use of sun beds in Sweden is currently proposed. In Sweden, sunbed use among 15-19 year-olds in Stockholm has declined (172). In 1993, 70% of females, and 44% of males used sunbeds and in 1999, 45% and 19% respectively. In 2013, 26% of 16-year-old girls and 3% of 16-year-old boys in Sweden had utilized a sun bed (173).

The Swedish Radiation Safety Authority (Strålsäkerhetsmyndigheten, SSM) is responsible for the Environmental Objectives (Sveriges miljömål) to reduce the number of skin cancer in Sweden. Since 2005, SSM have performed annual surveys of sun habits in Sweden. Further initiatives are the mobile application "Min Soltid" assessing the individual sun safety exposure and a pamphlet containing suggestions for children's outdoor environments (162). The book "En bok om solen" by Pernilla Stalfelt has been distributed to all schools with children in the second grade. The book gives fanciful illustrations of smart ways for children to become aware of risks with excessive sun exposure.

"Short shadow- the sun is strong. Long shadow – the sun is not so strong"

Illustration by Pernilla Stalfelt from "En bok om solen"

Observational intervention studies designed to improve sun protection practices and sunscreen use in children have to a large extent failed to demonstrate significant changes in naevi prevalence (e.g. (174, 175)). The hitherto only randomized clinical trial to determine whether the use of sunscreens attenuated the development of naevi in children was performed by Gallagher et al. in 2003 (176). After the 3-year trial, results demonstrated that children in the sunscreen group had developed fewer naevi than the children in the control group.

1.6.3 Current trends in sun tanning and sun protection

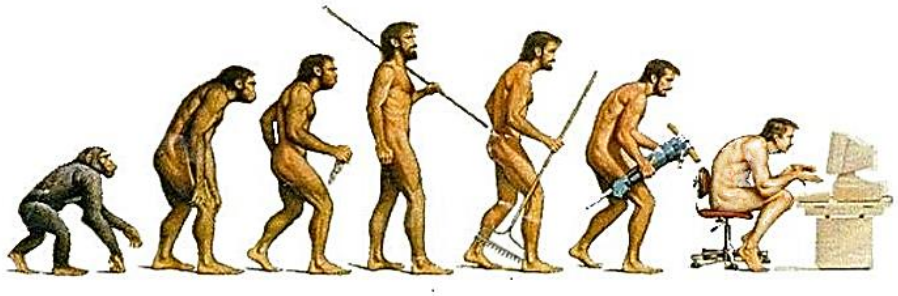
Limiting solar exposure is the most reasonable precaution for reducing the number of skin cancers. If only knowledge would determine protective behaviour, skin cancer prevention would be simple, but old habits die hard (177, 178). In a Danish prospective case-control study melanoma patients were followed during three consecutive years after melanoma diagnosis (179). The patients and matched controls were provided with dosimeters measuring daily UV dose from the first until third summer. The melanoma patients gradually increased their daily UVR dose until reaching same as levels as their controls. Thus, patients did not maintain the cautious sun behaviour they exhibit just after melanoma diagnosis. In a large survey performed among 2615 adolescents in Sweden 1996, there was evidence that those with higher knowledge of risks involved with excess sun exposure were more likely to be frequent sun bathers (143).

Still, there are an increasing number of reports, mainly from Australia, demonstrating improvements among children, adolescents and adults regarding attitudes towards intentional tanning, sunscreen use and wearing of long sleeved clothes (160, 180). A decline in the incidence of melanoma in young Australians has been detected and the findings are suggested to be due to improvements in sun prevention measures (49, 50, 181).

In Sweden, the Environmental Health Reports convey the results from questionnaire surveys regularly performed in Sweden. Between years 2003 and 2011 there were improvements

regarding use of various sun protection (173). For example in 4-year-olds, seeking shadow during mid-day was frequently used by 33% in 2003 and by 55% in 2011 and the use of sunscreens had increased from 69% in 2003 to 82% in 2011. In 12-year-olds similar improvements were reported.

The Swedish Media Institute regularly report of an increase in the daily use of various media in very young children (139). The most common media is watching TV, but computer games are frequently used by 67% of the 2-5 year-olds and 96% of 5-9 year-olds. Among the youngest age groups (2-5 year-olds) there are no major differences between boys and girls but between 5-9-year-olds, 73% of boys but only 34% of girls regularly play computer or TV games in their spare time. Does the "internet revolution" contribute to fewer opportunities for sun exposure in children?



Modified graphic with courtesy of FunnyChix.com

D-vitamin and sun exposure

UVB radiation is essential for converting vitamin D to an active hormone involved in human bone health. The optimal vitamin D status is under debate as the methods for measuring have not been standardized and the concentration of bioavailable D-vitamin is influenced by individual and ethnic variations in binding proteins (182). Studies have indicated that the harmful DNA effects of UV radiation (measured by T=T dimers in urine) are unavoidable when obtaining an increase in vitamin D levels during sun holidays (183). At the same time, mouse studies have shown a protective effect of vitamin D against the harmful effects of UVB, thus indicating a partly counteracting effect (184). The association of vitamin D status and skin cancer has been assessed in several epidemiological studies (e.g. (185)) and non-melanoma skin cancer (basalioma and squamous cell carcinoma) patients are found to have higher levels, probably related to excessive sun exposure. No similar association with melanoma risk has been verified but serum levels of vitamin D seem to be inversely associated with Breslow thickness (186).

The Swedish Radiation Safety Authority informs that a fair-skinned individual during the Swedish summer will receive a sufficient daily dose of vitamin D by staying outside in the sun, exposing the face and arms for 15 minutes during mid-day. Individuals with dark skin will require higher doses of UV exposures. Sunscreens to a part impair vitamin D synthesis when used in the recommended amount of 2 mg/cm^2, but not significantly when 1.5 mg/cm^{2,} is used, a value that corresponds better to what users apply in real life conditions (187).

1.7 MONITORING OF SUN EXPOSURE

Identifying biomarkers that are indicative of individual UV susceptibility and accumulated UV burden is a challenge (188). The monitoring of sun exposure would ideally entail a holistic perspective taking into account personal sun sensitivity and efficacy of DNA repair systems. It should control for environmental factors such as latitude, altitude, season, time of the day and the implementation of protection by clothing, shade or sunscreens. Any one monitoring instrument that meets all these tasks does not presently exist and the assessment of sun exposure can be attempted by various approaches.

1.7.1 Questionnaire surveys

Most studies assessing UV exposure rely on self-reporting; either by diaries or by performing questionnaire surveys retrieving retrospective sun exposure data (189). Questionnaires have the advantages of being easy to administer, fairly cheap and non-invasive but the reliability of self-reporting is dependent on several types of biases, mainly recall bias and social desirability bias (190, 191). Test-retest reliability of questionnaires has shown sufficient stability for questions regarding sun tanning behaviour, sun burns and self-assessment of skin photo type (192) and this is one important measure by which the standard for questionnaire surveys can be evaluated. Studies that have correlated self-reported information with objective UVR exposure measurements have shown valid results (e.g. (193)).

1.7.2 Dosimeters

Polysulphone film dosimeters are most commonly employed to estimate UVR exposure from sunlight and are considered reliable (122). However, the dosage determined with a stationary or personal dosimeter does not take skin photo type or use of sunscreen and clothing into account (125). It can be difficult to compare measurements because the body position (e.g. shoulder, wrist and ankle) of the personal dosimeter employed will influence the proportion of the ambient exposure detected. There may also be practicality issues with attaching a devise to the body.

1.7.3 Thymidine dimers

For monitoring of short-term UV effects the identification and quantification of DNA photoproducts can be used (188). The analysis of serum or urine concentrations of T=T dimers can serve as a biomarker of total body burden of UV exposure as they have been proven to directly correlate with level of UV exposure in a linear mode of action (194, 195). The possibility of analysing urine samples, as opposed to blood samples, has facilitated the quantification of T=T dimers in children. T=T dimers are limited by their short half-life allowing for measurements optimally within 3- 5 days after UV exposure (196). The total amount of T = T may differ about 5-fold among subjects given an equal UV dose.

1.7.4 Manual counting of naevi

The quantification of naevi has been proposed a suitable proxy for sun exposure, also correlating with melanoma risk (8). As latency between sun exposure and melanoma development may last for decades, naevi prevalence, especially in children may serve as early biomarkers when assessing effects of sun exposure and sun protection. Naevi have a shorter time to inception compared with melanoma but the exact time frame from a critical level of sun exposure to inception is not known.

The International Agency for Research on Cancer (IARC) in 1990 presented a protocol for identifying melanocytic naevi (197). The aim was to improve validity and reproducibility between studies of naevi. The protocol defines naevi as well-confined brown to black pigmented maculae or papules being darker than the surrounding skin. Other brown-pigmented lesions such as freckles, lentigines, café-au-lait spots and seborrhoeic keratosis may pose diagnostic difficulties and naevi counting should therefore only be performed after suitable training. The counting of naevi ≥ 2 mm is recommended to minimize risks of misclassifying small freckles or lentigines as naevi. However, restrictions of naevi counts to certain size subset may reduce reproducibility (198). Manual counting of naevi according to the IARC protocol can be said to be the "gold standard" which ultimately will enable comparisons of naevi prevalences between different observers. However, even with a greater degree of standardisation, at least 10% of the variation in full body counts may be due to inter-observer variation (198).

1.7.5 Digital imaging of naevi and teledermatology

Analogue and digital imaging of naevi has long been used by clinicians for surveillance of patients with risk of melanoma and in later years dermoscopy images have become an important diagnostic tool (199-201). In recent years, the utilization of teledermatology and teledermatoscopy has evolved rapidly and with the widespread accessibility of smartphone and cell phone mobiles, the market of mobile teledermatology has emerged (202, 203). This technique uses a store-and-forward methodology, allowing the imaging to be separated from the diagnostic procedure, which then can be done remotely by a specialist in the field.

Teledermatology has allowed for expert evaluation of skin lesions in remote populations and in individuals whose general health aggravates travels and visits to a hospital clinic (204, 205). Studies have shown an agreeable accuracy in diagnosing various skin lesions (206, 207), good patient-acceptance (208) and a potential for a more rapid evaluation of malignant skin lesions (209).

Even though mobile teledermatology has been used for evaluation of individual naevi it has not been validated for displaying the overall numbers of naevi within a defined body site and its use in children is hitherto scarcely studied (210-212).

2 AIMS

The overall aim of this thesis was to study the influence of sun exposure on prevalence and localization of naevi in Swedish children and to evaluate their potential role as objective population biomarkers correlating with different sun exposure patterns and with melanoma.

Specific aims:

- To investigate the 5-year changes in sun travels, sunburns and sun-protective regimens in two consecutive populations of 7-year-old children and to assess how naevi prevalence in children correlate with reported changes in sun exposures.

- To investigate if mobile teledermatology could offer a valid methodology compared with standard manual, face-to-face counting of naevi on the back of children. Further, to evaluate the feasibility of mobile teledermatology to monitor naevi prevalence in children.

- To analyse whether the overall reduction in naevi prevalence demonstrated in 2007 among children in southern Sweden had impacted differently on body sites based on their main pattern of sun exposure. Further, to analyse the overall and body-site specific differences in naevi prevalences between boys and girls.

- To investigate if residing at different latitudes affects the anatomical distribution of melanoma in adults and naevi in children. Further, to analyse if body-site distribution and gender profiles of melanoma and naevi in children were concordant and whether there were differences between northern and southern Sweden.

3 MATERIALS AND METHODS

STUDY 1

In 2001/2002 a cross-sectional, population-based survey was initiated by Ylva Rodvall et al to investigate sun exposure patterns, travelling, parental sun protective regimens and numbers of naevi among children residing at different latitudes in Sweden. (16). The municipalities of Kiruna (67.8°N) and Piteå (65.3°N) in northern Sweden and Falkenberg (57.0°N) and Ljungby (56.9°N) in southern Sweden were selected. These municipalities were chosen based on their demography, comprising comparable numbers of 7-year-old children (born 1994) and socioeconomic parameters, such as income levels and unemployment rates and neither should hold a university. Further, they were set to represent an inland and a coastal location at either latitude.

Study I was designed as a 5-year follow-up study, performed in 2007, using the same setting and survey procedure as in 2002. Exclusively the two municipalities in southern Sweden were included in the follow-up in 2007. All 7-year-old children (born 1999) residing in Falkenberg and Ljungby were identified and their parents received a letter describing the study design and routines for clinical examination together with a questionnaire and a consent form.

Questionnaire

The questionnaire comprised questions regarding the child´s skin photo type according to Fitzpatrick's (65), the frequency in application of predefined sun protective measures such as sunscreens, clothing, staying in the shade and staying inside during peak sun hours. History of sunburns, episodes being naked in the sun and holidays spent in sunny resorts abroad were recorded within age intervals; < 2 years of age, 2-4 years of age and > 4 years of age. The parents also answered a question specifying to what extent they themselves fancied outdoor sun tanning.

Clinical examination

The same research nurse examined all children in 2002 and 2007. Before pursuing the initial study 2002 the nurse was trained and validated against an experienced dermatologist with a strong agreement calculated by κ coefficient = 0.86 (95% confidence interval 0.71-0.93) in 100 children (16). The nurse performed height and weight measures of each child. The pigmentary phenotype, i.e. child's hair colour and eye colour was also assessed by the nurse. The counting of naevi followed the IARC protocol for identifying melanocytic naevi (197). The total number of naevi with a diameter ≥ 2 mm was counted, with exclusion of the scalp, genitalia, buttocks and abdomen below the umbilicus. Naevi were classified into 2 groups; ≥ 2mm to < 6mm and ≥ 6mm utilizing a Plexiglas overlay as a measuring aid.

STUDY II

Study II was set in the Pediatric Dermatology outpatient clinic at Karolinska University Hospital, Solna. The Pediatric Dermatology outpatient clinic receives nearly 3000 referrals per year from general practitioners, paediatricians or other specialists in dermatology. The clinic investigates children with an extensive variety of both common and exclusive inflammatory skin diseases, vascular and pigmented skin tumours, skin infections and genodermatoses.

Children eligible for inclusion in Study II were aged 7 to 16 years of age with skin photo type I-IV according to Fitzpatrick´s (65). Children younger than 7 years were not included because

they were expected to have a low number of naevi on the back. Children and accompanying parents were invited to take part in the study in conjunction to their scheduled visit attending the Pediatric Dermatology outpatient clinic between May 2012 and January 2013. Selection of eligible cases was based upon whether time during visit allowed for participation and was therefore not consecutive.

One dermatologist performed the manual counting of naevi and digital imaging exclusively on the child´s back. The back was chosen for practical reasons, being easy accessible and offering a two-dimensional surface anatomically suitable for photographic imaging. In addition, the density of naevi on the back correlate well with whole-body CMN counts (213, 214) and is a site of biological interest by being subjected to intermittent sun exposure which associates with higher risk of both naevi and melanoma development (136).

The boundaries of the back were defined by the anatomic paper chart used in Study I and IV (see Figure 2) which covered the area from the nape of the neck, including the shoulders and down to the iliac crest. To facilitate the remote size assessments from the digital images, a small sticker with a millimetre scale was placed adjacent to a nevus approximately 2 mm of size depicted as an "index-nevus". The location of each naevus on the back was recorded on the anatomical paper chart. A plastic template was used to categorise naevi into sizes < 2 mm, ≥ 2 till < 6 mm and ≥ 6 mm.

The digital photographic imaging was performed using an iPhone 4s mobile phone comprising an 8.0 megapixel camera downloaded with the mobile application Dermicus. Dermicus is developed at Karolinska University Hospital and is used for dermatological consultations between primary health care givers and selected dermatologists (215, 216). It is used as an interactive telemedicine system with the possibility to attach a dermatoscopic device for closer assessment of individual skin lesions. The application Dermicus is CE marked and has a safely-coded login system, sending all images encrypted to an external server which is certified for high security storage.

A total of four images were taken, two for overview over the back (Figure 1) and two including a close-up of the index-naevus. The examination rooms were equipped with standard fluorescence light in the ceiling and, whenever present, daylight from the window. The camera was set in autofocus mode and was used without flash or zooming. Imaging was performed at a distance which included the area just outside the back area selected for counting naevi.

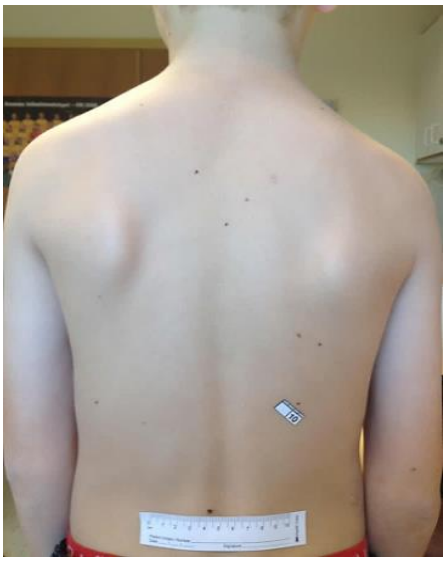


Figure 1. Example of a digital image of child's back using an iPhone 4s mobile phone camera

Two other dermatologists independently viewed the digital images. They were blinded to each other's result as well as to the results of the manual counting. The monitoring process was standardized by pasting the images in Microsoft PowerPoint and then viewing them in the slideshow mode. A 19-inch computer monitor with resolution set to 1280x1024 and a well-cleaned screen was used.

Both dermatologists counted the total numbers of naevi on the back and also estimated the size; < 2 mm, ≥ 2 to < 6 mm and ≥ 6 mm, using the index naevus as a reference. The location of each naevus was noted on a paper chart, same as used for the manual counting. Presence of any skin eruptions, e.g. excoriations, scars, eczema or acne, was commented and indicated directly on the paper charts. A quality rating of the images on a five-level ordinal scale; very good, fairly good, neither good nor bad, fairly bad, very bad, was also recorded.

STUDY III

The results from Study I had demonstrated an overall significant reduction in naevi densities over a 5-year period among 7-year-old children residing in southern Sweden. In Study III the same two age-standardized populations (n=1189 corresponding to77.6 % response rate in both years) of children were sub-analysed regarding the changes in naevi densities per body-site and in boys and girls, respectively.

In both year 2002 and 2007 the location of each individual naevus had been recorded on an anatomic paper chart divided into 16 body sites (A-P) as originally presented by Augustsson et al. (217) (see Figure 2). All naevi with a diameter ≥ 2 mm, with exclusion of the scalp (area B), genitalia/abdomen below the umbilicus (area H) and buttocks (area J) were counted.

Body sites were merged into four major body sites: the face, arms, trunk and legs and were also classified according to their main pattern of sun exposure; chronic, intermittent or rare. The face and dorsum of hands were considered chronically sun exposed. The trunk, arms, legs and dorsum of feet were considered intermittently sun exposed and the medial aspect of the arm, the palms and soles as rarely sun exposed.

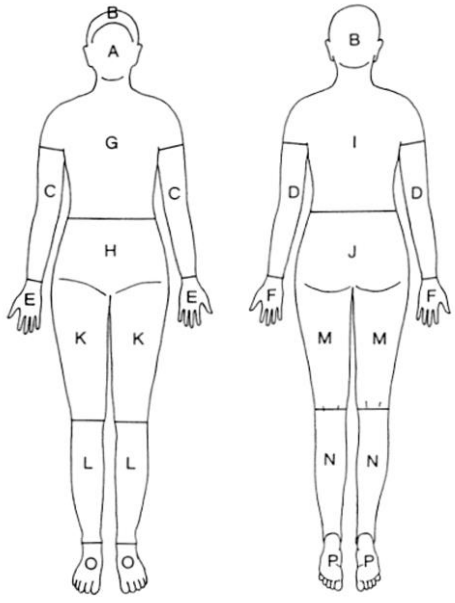


Figure 2. Anatomical paper chart used for recording common melanocytic naevi on 16 body sites (A to P). Published with courtesy of Dr. Agneta Augustsson.

STUDY IV

Study IV was designed as a large cross-sectional population-based study set to investigate the anatomical distributions of melanoma in northern and southern Sweden compared with sub-site distributions of naevi in children residing within the same geographic regions (Figure 3).

Northern Sweden was represented by the population residing within the North Health Care Region (counties of Norrbotten, Västerbotten, Jämtland and Västernorrland). Populations in southern Sweden were represented by the South, South-East and West Health Care Region (counties of Skåne, Kronoberg, Blekinge, Halland, Östergötland, Jönköping, Kalmar and Västra Götaland). The regions were selected to geographically cover the municipalities surveyed for naevi in children, to provide substantial numbers of melanoma and to represent both coastal and inland regions.

Geographic characteristics and population variables were extracted from Statistics Sweden (218) and climate parameters were from the STRÅNG data base held by the Swedish Meteorological and Hydrological Institute (SMHI) (26, 219).

Figure 3. Northern Sweden represented by the North Health Care Region (blue) and southern Sweden by the three most southern Health Care Regions (red) covered data on incidence and body-site localization of cutaneous malignant melanoma 1990-2012.

Black-marked regions on the map indicate the four municipalities (Kiruna and Piteå in the north and Ljungby and Falkenberg in the south) engaged in the cross-sectional naevi studies performed among 7-year-old children in 2002.

The Swedish Cancer Register (SCR)

The numbers and incidence rates of invasive melanoma per body site from 1990 through 2012 were obtained from the open access cancer statistic data base held by The National Board of Health and Welfare (220). This database is linked to Swedish Cancer Registry (SCR). The nationwide SCR was founded in 1958 and has compulsory reporting by both the clinician and histopathologist responsible for diagnosing a malignant tumour. This double-reporting ensures virtually all incident cases of cancer in Sweden to be registered and SCR serves as a comprehensive, high quality data register for cancer incidence in Sweden.

Four major anatomical sites were selected based on coding according to the 10th revision of International Classification of Diseases (ICD-10): face, upper extremities, the trunk and lower extremities. Sites with multiple melanomas or unspecified location were excluded as were melanomas on the scalp (including the neck) because this site lacked information on

childhood naevi for comparison. Each melanoma reported to the SCR was treated as a unique case, thus the numbers of melanoma did not per se correspond to same numbers of individuals. Melanoma data were stratified by sex (male and female) and in age groups (0-29, 30-49, 50-69 or 70+ years) as it is known that melanoma incidence varies with age.

The Swedish Melanoma Register (SMR)

All data regarding histogenetic characteristics of melanomas in northern and southern Sweden were compiled from two reports from SMR; the National Quality Register for Skin Melanoma 1990-2008 and 2009-2012 (12, 13). Melanoma subtypes included lentigo maligna melanoma (LMM), superficial spreading melanoma (SSM), nodular melanoma (NM) and acral lentiginous melanoma (ALM). Level of invasion of the melanomas was based on Clark level and Breslow thickness.

The SMR is a nationwide register founded in 2003 on initiative of the Swedish Melanoma Study Group with the aim to provide a scientific basis for a standardized high quality care of melanoma patients in Sweden, including prevention, diagnostic activities and treatment. The SMR comprises detailed variables related to diagnostics and management of melanoma and is based on prospective reporting from each of the six regional cancer centres in Sweden. The SMR covers over 97% of all melanoma cases in Sweden.

Naevi distributions in children

The numbers and densities of naevi per body-site in children living in northern and southern Sweden were obtained from the population-based study performed in 2001/2002 among 7-year-old school children residing at different latitudes in Sweden (details previously described in Study I) (16). In Study IV, all 7-year-old children with skin photo types I-IV (n=1360 corresponding to 81.1% of the eligible population) residing in the two municipalities in northern Sweden: Kiruna 68°N and Piteå 65°N and in southern Sweden: Ljungby 57°N and Falkenberg 57°N were included.

Body-site distributions of naevi ≥ 2 mm were presented both as crude numbers and as mean densities per body-site (calculation of naevi densities described in Statistical analyses). Merging into same four major body regions as for melanoma was performed: face, upper extremities, the trunk and lower extremities. For the comparison of anatomic distributions between melanoma in adults and naevi in children in northern and southern Sweden the crude numbers were presented as relative proportions (%) out of total body area (100%). The distributions of melanoma, stratified by sex and age group on the four major anatomical sites were compared with naevi distributions using the chi-square (χ^2) goodness-of-fit statistics.

4 STATISTICAL ANALYSES

Calculation of mean and median naevi densities

Calculation of naevi densities takes into consideration the variation in total body surface area (BSA) between individuals and between different body sites. To calculate total naevi densities in Study I, III and IV, the BSA was calculated from height and weight of each child using Mosteller simplified formula (221). The BSA in 8-10 year-olds is approximately 1 m^2 meaning the naevi densities correspond quite well to the total numbers of naevi. For comparison, BSA in adults is approximately 1.9 m^2 in men and 1.7 m^2 in women.

In Study III and IV the number of naevi/m^2 BSA were computed separately for the 16 body sites using burn area estimation charts modified for children by Lund CC & Browder NC (222). Regional BSAs for 7-year-olds were estimated as mean of 5 and 10 years.

The overall distribution of naevi and naevi densities in children of the same age is not normally distributed. Instead it is naturally skewed to the right. This means that most individuals have a low to medium number of naevi while a few individuals have very extensive numbers. In Study III, where smaller body parts were investigated for numbers of naevi this was accounted for by presenting both means (with standard deviations) and median values (with 95% confidence intervals). Additionally, the proportion (%) of children having at least one naevus within the different body sites was presented.

Poisson regression model

In Study I the numbers of naevi in each year of 2002 and 2007 were adjusted for subject-characteristic variables (e.g. gender, skin photo type, hair colour and eye colour) and questionnaire variables first using univariate analysis. All variables with a p-value of 0.10 or less in the univariate analysis were considered for inclusion in the multivariate Poisson regression model. In case of overdispersion, specification of a scale parameter was used to fit the Poisson distribution. This approach enabled definition of variables that were significantly associated with changes in naevi density.

Weighted kappa analysis

The kappa coefficient is used for comparing agreements between two raters or two methods (223, 224). The weighted kappa analysis allows for assessing disagreements differently and is especially useful when categories are ordinal. In Study II, a Cohen's kappa analysis weighted according to Cicchetti-Allison was used. Strength of agreement according to Landis and Koch for value of kappa was set to: ≤ 0 Poor, 0.01–0.20 Slight, 0.21–0.40 Fair, 0.41–0.60 Moderate, 0.61–0.80 Substantial, and 0.81–1.00 Almost perfect agreement.

Negative binominal and zero-inflated negative binominal tests

In Study III the statistical tests for comparing changes between year 2002 and 2007 and between boys and girls were performed using relative risks calculated with zero-inflated negative binominal test or negative binominal test as appropriate. These analyses are used when there is an excess of null values as was seen for many of the smaller body sites. The relative risk is a ratio between a reference category, with a value set as 1.0, and the category that is to be studied. Given that the 95% confidence intervals do not overlap 1.0, the relative risk is significant and also gives an easily interpretable value of the magnitude of difference from the reference category.

Age-standardized incidence rates of melanoma

In Study IV the incidence rates of melanoma per 100 000 were age-standardized according to population in Sweden year 2000. Standardization is necessary when comparing populations that differ with respect to demography because age has a powerful influence on the risk of cancer.

Chi-Square Goodness of Fit Test

The Chi-Square Goodness of Fit analysis is statistical model that describes how well the distribution of observed values fits with the values expected. If the computed test statistic χ^2 is large, then the observed and expected values are not close and the model is a poor fit to the data (225). The number of degrees of freedom is the number of values in the final calculation of a statistic that are free to vary and is calculated by n-1 where n is the number of values (or categories). In Study IV it was tested whether the observed distribution of melanoma (face, trunk, upper limbs, and lower limbs), stratified by geographical region, sex and age category was comparable with distributions of naevi in children (treated as the expected values).

5 ETHICS APPROVALS AND CONSIDERATIONS

All studies in this thesis were approved by the Regional Ethical Review Board in Stockholm, Sweden and the parents had given informed consent for the participation of their children (Dnr: 01-182, 2006/1466-31/2, 2007/1177-32 (Study I, III, IV) and Dnr: 2012/225-31/3 (Study II)).

The clinical naevi investigations in 2002/2002 and 2007 were performed in collaboration with the school staff at each of the schools in the selected municipalities. Most often the examinations took place in the local school nurse's office, a setting chosen for convenience but also to ensure a safe and well-known environment for the children. The parents were invited to participate at the clinical examinations and at any point ask questions, but this opportunity was however very rarely utilized. The children who participated were most often represented by a whole class of first-year pupils.

The study was designated to include children with fair skin photo types (I-IV) as these are risk populations for having many naevi which correlate with increased melanoma risk. This could potentially have raised ethical considerations and was handled by counting naevi in all participating children indiscriminately of their skin colour and excluding data for children with darker skin types in retrospect.

When performing the counting of naevi, certain body parts were excluded. The scalp was excluded for practical reasons as identifying naevi in this region is obstructed by hair. Naevi on genitalia, buttocks and abdomen below the umbilicus were not counted so that all children could keep their underwear on, not to risk embarrassing or intimidating the children.

Study II was a clinical study performed at the Pediatric Dermatology Department at Karolinska Hospital in Solna, validating the novel use of mobile teledermatology for estimating prevalence of naevi on the back of children. When sending out the scheduled appointment time to meet with a dermatologist, the parents were given written information presenting the study. They were informed about the procedure of manual counting of naevi and the digital imaging. Some of the children and accompanying parents were informed about the study in direct conjunction to their scheduled visit.

During the manual counting of naevi and imaging procedure, no child expressed reluctance or withdrew their consent to participate. Girls wearing a brassiere could keep this on after securing that no naevus was covered by the straps. The rapidly expanding use of internet as a major social medium alerted us to the issue of safeguarding personal integrity when transmitting and storing the digital images. We had anticipated some concern or questions and children and parents were encouraged to express any ambiguities. However, overall positive responses towards using a modern technique for research purposes were expressed. This might however largely be due to the fact that the survey was set in a university hospital clinic, among an urban population and the presence of an accompanying parent likely contributed to a high participation rate. If performing similar studies in non-hospital settings these questions might be more of a concern.

6 RESULTS

STUDY I

A total of 1534 eligible 7-year-old children residing in the selected municipalities in 2002 and 2007 were identified. The final number of children enrolled were 1190 (681 in 2002 and 509 in 2007) which corresponded to a final response rate of 77.6 %.

The results from the parental questionnaires demonstrated overall significant improvements in use of child´s sun protective regimens over the 5-year period. Between 2002 and 2007 parents reported more frequent use of sunscreen (+29%), clothing (+30%), staying in the shade (+123%) or indoors (+136%) during peak sun hours. On average 2–3 times as many children in 2007 were reported as never having been naked, or partly naked, in the sun before 4 years of age. Yet, travelling on sun holidays abroad before the age of 2 years had almost doubled and sunburns were reportedly as frequent in 2007 as in 2002, e.g. nearly 70% of children had experienced at least one sunburn after 4 years of age.
The parental responses to the question as to what extent they themselves fancied outdoor tanning had not changed significantly between 2002 and 2007 and on average 70% reported they fancied tanning 'very much' or 'fairly much'.

Clinical data of participating children revealed that having blond/ash blond hair colour (> 80%) and blue/grey eye colour (> 70%) and skin photo type III (> 60%) was the most common complexion in both years. Even though more children had dark hair colour in 2007 (13.8%) versus 2002 (7.3%), the parents in 2007 more frequently reported their children having a sun sensitive skin photo type (I and II) in 2007 (25.8%) versus 2002 (14.2%).

Analysing the prevalence of naevi expressed as the mean number of naevi per square metre BSA demonstrated that naevi densities were significantly lower in 2007 (8.6 (95% CI 8.0–9.2)) compared with 2002 (12.8 (95% CI 12.2–13.5)) which corresponded to an overall reduction by 33%.

A multivariate analysis was performed to assess all clinical and survey data with a potential to affect naevi counts. The year of examination was the strongest predictive factor. Other significant predictors were hair colour, the numbers of sunburns > 4 years of age and ever having spent vacation in a sunny seaside holiday resort abroad > 4 years of age. Blond and ash-blond hair colours were associated with the highest naevi counts and red hair with the lowest.

STUDY II

A total of 114 children and their parents were approached for inclusion in the study, 109 accepted and 5 declined. In 12 separate cases (11%) the transmission of the digital images from the mobile application to the external server failed which rendered exclusion. Final statistical analyses were based on 97 children (41 boys, 56 girls). Median age was 11 years (range 7-16).

The mean number of naevi of all sizes adjusted for body surface area were comparable between manual counting (9.4 (SD 12.5)) and the 2 dermatologists' counting of naevi from digital images (10.6 (12.3) and 9.6 (13.7), respectively).

The inter-method reliability for total number of naevi of any size on the back showed substantial agreement for both dermatologists when compared with the manual counting: dermatologist 1 (κw = 0.69 (0.57–0.81 [95% CI]) and dermatologist 2 (κw = 0.78 [0.70–0.86]). Inter-rater reliability was also substantial (κw = 0.80 [0.73–0.87]) between the 2 dermatologists.

When analysing the 3 naevi size categories (< 2 mm, ≥ 2 to < 6 mm and ≥ 6 mm) separately the ratings differed slightly: naevi < 2 mm demonstrated fair agreement for dermatologist 1 (κw = 0.28 [0.17–0.38]) and moderate agreement for dermatologist 2 (κw = 0.55 [0.46–0.64]) versus the manual assessment. For naevi size ≥ 2 to < 6 mm the agreement was moderate for dermatologist 1 (κw = 0.54 [0.39–0.70]) and substantial for dermatologist 2 (κw = 0.68 [0.56–0.80]). Inter-rater reliability was moderate for naevi < 2 mm (κw = 0.49 [0.37–0.61]) and substantial for naevi ≥ 2 to < 6 mm (κw = 0.64 [0.54–0.75]). Naevi ≥ 6 mm were very few and were not considered for the agreement analysis.

In an additional analysis (not presented in the published manuscript) an "intra-rater, inter-method reliability" analysis was performed. Two months after the manual counting of naevi in all children was completed, the dermatologist counting manually also viewed and assessed naevi from the 97 digital images. This complementary analysis demonstrated a weighted kappa value for the total numbers of naevi (κw = 0.77 [0.76–0.87]) corresponding to substantial agreement; a result well comparable with the two dermatologists who exclusively viewed the images.

The quality of the images as judged by the two dermatologists ranged from "very good" to "very bad". The majority; 74% and 64% respectively, were noted as "fairly good" (Figure 4). None of the images had to be excluded from analysis due to too poor quality making the identification of naevi impossible.

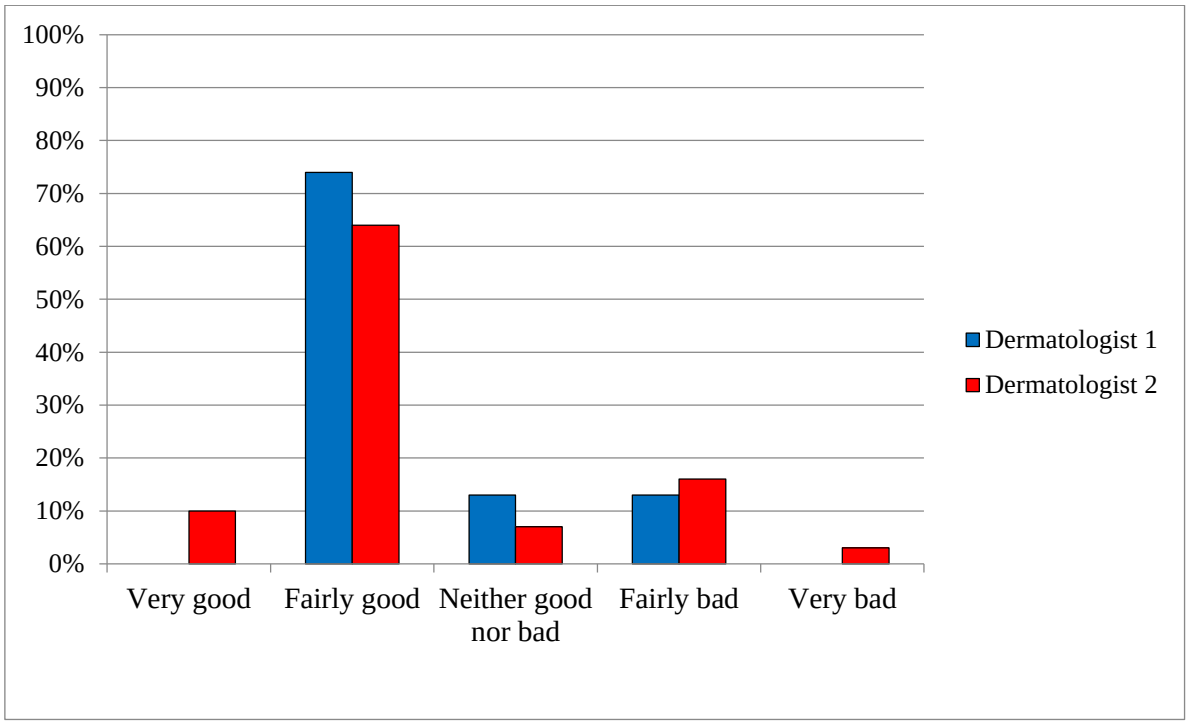


Figure 4. Quality of the 97 digital images as judged by the two dermatologists

STUDY III

Between 2002 and 2007 the boys had had the largest reduction by a total median number of 5 naevi (from 12 to 7 ($p< 0.0001$)) and the girls by a total median number of 3 naevi (from 9 to 6 ($p< 0.0001$)).

Boys had higher median naevi densities on the face and trunk in either year of 2002 and 2007 compared with girls and girls had slightly higher densities on the legs in both years. These gender profiles did appear even more evident in 2007.

In 2002, the median naevi densities on the intermittently sun exposed body sites were generally higher than on the chronically sun exposed sites: 13.8 (95% CI 8.0-22.7) compared with 11.0 (0.0-20.5). In 2007, naevi densities were lower on the intermittently sun exposed body sites: 8.7 (4.7-15.2) compared with 10.3 (0.0-14.4) on chronically exposed sites.

Table 2. Relative risks (95% confidence interval) for number of naevi by major anatomic site and by main type of sun exposure among 7-year-old children in year 2002 and 2007

		2007 vs 2002 Boys	2007 vs 2002 Girls	2007 vs 2002 All
Total body	Relative risk[a] (95% CI) p-value	0.66 (0.59-0.74) **<0.0001**	0.70 (0.62-0.79) **<0.0001**	0.68 (0.63-0.74) **<0.0001**
Major anatomic site				
Face	Relative risk (95% CI) p-value	0.99 (0.74-1.31) 0.9224	0.83 (0.57-1.20) 0.3099	0.92 (0.77- 1.10) 0.3643
Arms including hands	Relative risk (95% CI) p-value	0.63 (0.51-0.77) **<0.0001**	0.89 (0.73-1.08) 0.2308	0.73 (0.64- 0.84) **< 0.0001**
Trunk	Relative risk (95% CI) p-value	0.74 (0.66-0.84) **<0.0001**	0.77 (0.67-0.88) **0.0002**	0.75 (0.68-0-82) **< 0.0001**
Legs including feet	Relative risk (95% CI) p-value	0.43 (0.35-0.54) **<0.0001**	0.53 ((0.44-0.64) **<0.0001**	0.48 (0.42-0.55) **< 0.0001**
Main type of sun exposure				
Chronic[b]	Relative risk (95% CI) p-value	1.03 (0.85-1.25) 0.7775	0.91 (0.67-1.25) 0.5655	0.99 (0.84-1.17) 0.9313
Intermittent[c]	Relative risk (95% CI) p-value	0.63 (0.57-0.71) **<0.0001**	0.69 (0.61-0.79) **<0.0001**	0.66 (0.61-0.72) **< 0.0001**
Rare[d]	Relative risk (95% CI) p-value	0.69 (0.50-0.93) 0.0169	1.02 (0.78-1.03) 0.8968	0.86 (0.69-1.07) 0.1733

[a] Relative risks calculated with zero-inflated negative or negative binominal test including 95% CI (confidence intervals). Bonferroni corrected p-values below 0.00125 (considered statistically significant) marked in bold.
[b] Chronically sun exposed body sites includes the face and dorsal of hands
[c] Intermittently sun exposed body sites includes lateral aspect of arms, chest, back, anterior and posterior aspects of thighs and lower legs and dorsal feet
[d] Rarely sun exposed body sites includes medial aspect of the arm, palms and soles

The statistical analyses verified that the number of naevi on chronically sun exposed sites had not changed significantly between years; relative risk 0.99 (95% CI 0.84-1.17, p= 0.9313) (Table 2). However, on intermittently sun exposed sites a highly significant reduction of naevi densities was demonstrated; relative risk 0.66 (0.61-0.72, p< 0.0001). Number of naevi on rarely sun exposed body sites did not differ significantly between years. As naevi on body areas that are generally strictly covered from the sun (lower abdomen/ genital area and buttocks) was not counted, the rarely sun exposed category was considered less valid.

STUDY IV

The descriptive analysis regarding climate factors in northern and southern Sweden demonstrated that these were substantial with a 51.4% higher average yearly sum of CIE UV irradiance (mWh/m^2) and a 6.2 degrees Celsius higher annual mean temperature in southern Sweden (Table 3). Socioeconomic status assessed as mean disposable income and highest level of education demonstrated slightly higher disposable incomes and education level in southern Sweden.

Table 3. Geographic characteristics, demography, and climate conditions in northern and southern Sweden

Geographic region	Northern Sweden	Southern Sweden
Latitude (^{0}N)	62-69	55-58
Area (km^2)	225 121	82 953
Population (n)[a]	880 035	4 570 184
Mean age in population (years)[a]	42.1	41.4
Life expectancy (years) [a]		
Women	83.0	83.8
Men	78.9	80.0
Mean disposable income (1000 SEK) [b]	170.8	177.1
Highest level of education (%)[b]		
Elementary school (%)	10.1	13.8
Upper secondary school 2 years (%)	31.9	25.4
Upper secondary school 3 years (%)	22.3	22.0
University education less than 3 years (%)	13.9	15.0
University education 3 years or more (%)	19.9	22.0
Graduate education (%)	0.9	1.1
Missing data (%)	1	0.7
Proportion with foreign origin (%)[b]	7.6	13.7
Average yearly sum of CIE UV irradiance (mWh/m^2)[c]	75529.4	114364.6
Annual mean temperature (C)[d]	0.6	6.8
Annual mean sun hours (n)[d]	1518	1727

[a] Statistics Sweden 2013 [b] Statistics Sweden 1997-2013 [c] CIE UV, ultraviolet radiation weighted according to the Commission Internationale de l´Eclairage 1999-2013 [d] Swedish Meteorological and Hydrological Institute 1961-1990

Melanoma incidence rates in northern and southern Sweden demonstrated a two-fold higher incidence (40.5/100.000 age-standardized population) compared with 19.6/100.000 in northern Sweden in year 2012.

For the study period 1990-2012 the mean incidence of melanoma per body site was highest on the trunk, followed by the upper extremities, lower extremities and face in men. In women the highest incidence of melanoma was found on the lower extremities, followed by the trunk, upper extremities and face.

The melanoma subtypes were to a larger extent SSM (56.9% versus 53.8%) and LMM (6.4% versus 5.4%) in southern Sweden. In northern Sweden larger proportions were NM (24.0% versus 19.8%) and more melanomas had higher level of invasiveness according to Breslow thickness and Clark level.

The total mean naevi densities were overall lower in children residing in northern Sweden: 7.3 (SD 5.4) in boys and 7.0 (4.7) in girls in the north versus 13.3 (8.4) in boys and 11.9 (8.5) in girls in the south. The highest mean density of naevi was found on the trunk in both boys and girls. Girls in both northern and southern Sweden had higher mean naevi densities on the legs compared with boys and this matched with melanomas in women being more prevalent on the legs.

The relative proportions of melanoma per anatomic site recorded for the face, upper extremities, trunk and lower extremities demonstrated largely similar distribution patterns in northern and southern Sweden. A slightly larger proportion of melanomas were located on the trunk in southern Sweden and on the face in northern Sweden. With increasing age, more melanomas arise on the face, mainly at the expense of lower extremities in men and the trunk and lower extremities in women. In the 0-29 year age group most melanomas in southern Sweden were located on the trunk while on lower extremities in northern Sweden. This was in practice imputed by melanomas diagnosed between 15 and 29 year olds as a subanalysis demonstrated that the number of melanomas diagnosed prior to 15 years was very low (2 cases in the north and 16 cases in the south 1990-2012).

The body-site proportions of naevi in children were very similar in northern and southern Sweden but, in concordance with melanoma, a tendency for more truncal localisation in southern Sweden whilst more on the face in northern Sweden. The distribution of naevi in 7-year-old boys in northern Sweden matched significantly with melanoma in men in the 30-49 and 50-69 year age categories. For boys in southern Sweden, significant agreements were seen for the 0-29, 20-49 and 50-69 year age categories. Among females, the anatomic distribution of naevi in girls matched less well with melanoma in women when compared with the male counterpart. However, in southern Sweden the distribution of naevi in girls was significant and border-line significant, respectively, when compared with the distribution of melanoma in women in the 0-29 years and 30-49 years melanoma age categories.

7 DISCUSSION

STUDY I and III

In Study I and III, all naevi data and the questionnaires were obtained from population-based, cross-sectional studies. These are examples of observational studies which by their nature hold both strengths and limitations. Observational studies can be subjected to several biases and they generate hypotheses rather than establish causal relationships.

The foremost strengths in the clinical examinations were the use of the same methodology, the same research nurse and adhering to the IARC naevi protocol (197) in all studies. This gave a routine and a standardisation of the naevi counting process. Prior to the start of the surveys, the nurse was validated against an experienced dermatologist for the ability to discriminate naevi and for the ability to exclude other pigmented lesion [kappa coefficient = 0.86 [95% confidence interval (CI) 0.71-0.93]. This approach provided reliable naevi counts and created high internal validity, i.e. ensuring that the variables set to be measured were correctly measured. Naevus counting will always pose some uncertainty due to the fact that some pigmented lesions may be clinically impossible to discriminate from true naevi. For example, it is still not known whether lentigines can evolve to become naevi or if they represent separate entities (226). By using the same rater for all naevi examinations, the risk of introducing systematic errors (bias) in the counting process was minimized.

The population-based sampling approach, together with the retrieval of a high response rate for participation, means the results provided substantial external validity, meaning the results can be said to be representative of the population surveyed. Even so, the studies were performed solely in fair-skinned children and results may not be applicable for more dark-skinned children, although they were not considered as risk populations in these studies.

The pigmentary characteristics recorded in 2002 and 2007 where largely homogenous between the populations but more children were dark-haired in 2007. It could be speculated that this was because more children in 2007 had a non-Scandinavian ancestry as indicated by data from Statistics Sweden for the study period. However, over the same 5-year-period, eye colour had not changed significantly and having skin photo type I or II was significantly more prevalent in 2007. The interpretation of these differences and how they might have influenced naevi prevalence is complex. Studies from US of dark-haired children with fair photo skin types have indicated that they might even have higher naevus counts compared with more light-haired children with same skin photo type (227) which would contradict a reduction in numbers of naevi based solely on differences in basic phenotypic characteristics. The assessment of skin photo type was based only on the parents' reports. It might be speculated that because the children in 2007 had been more shielded from the sun they were perceived by the parents as being more sun sensitive and maybe their ability to tan was not known.

The reasons for non-participation in the studies 2002 and 2007 were not possible to fully assess as participation was optional. However, some parents replied that their child had no naevi, and asked if they then still needed to be examined. If this was a common reason for the parents not to let their child take part in the study, selection bias could have been introduced. This would then slightly have overestimated the naevi prevalence in the population but would however not likely have differed between year 2002 and 2007.

The questionnaire used in the surveys in 2002 and 2007 were constructed to investigate parental sun protective regimens, sunburns and travelling among Swedish children. The questions were based on similar well-acknowledged international surveys from reputable research groups to ensure comprising the key determinants known to influence naevi counts

in children. The reporting was naturally restricted to a maximum of the previous 7 years and it is likely to assume that the reporting was reliable due to the reasonably short time period to recall, but this may have varied depending on the question. For example, having spent a sun holiday abroad had a larger proportion of non-reporting compared with other questions.

Societal desirability bias could potentially be an issue when approaching parents, being the guardians of their child's sun safety. This could not be ruled out but there are no indications as to believe this would differ between year 2002 and 2007. Interestingly, the parents readily reported (only 0.9% non-reporting) that the majority fancied outdoor tanning very or fairy much in both 2002 and 2007. Thus, even if the parents themselves enjoyed outdoor tanning as much in both years, they still protected their children more stringent in 2007.

The proportion of children that had experienced at least one episode of sunburn by the age of 7 years was 68.8% and 68.6% in 2002 and 2007, respectively. This was by us perceived as a rather high number, well comparable with reports in children of the same age in Denver, Colorado (69%) (136). The question of sunburn in our study was addressed by asking "How many times has your child experienced sunburn? (pain and redness of the skin)" Comparing number of sunburns in-between studies could be spurious due to different wording of the question, in some studies comprising "blistering" to the definition of sunburn. A positive interpretation of the numbers of sunburns reported in 2007 was that these had not increased, even though travelling on sun vacations had become more prevalent.

STUDY II

Manual counting of naevi has hitherto been the standard procedure when performing nevus surveys among children. Face-to-face consultations give the advantage of tactile information aiding the eye when discriminating naevi from other pigmented skin lesions. Also, the presence of any skin rash or scars would supposedly be more easily accounted for when performing manual counting of naevi. With this in mind, in Study II we wanted to evaluate how digital imaging would perform in comparison with manual counting of naevi. A few previous studies have used viewing of naevi by analogue, digital and/or dermoscopic imaging of naevi but validation studies were lacking and the use of remote assessment of naevi prevalence using mobile teledermatology was not previously tested.

We perceived that the counting of naevi from digital photographs would risk misclassification, both of true naevi being assessed as other skin lesions and of, for example, excoriations or crusts that could imitate naevi when viewed on a screen. The risk of both differential and non-differential misclassification was to some extent addressed by using two independent raters viewing the images. They were blinded to each other's results and to the manual counting but had prior to the investigation performed joint sessions to set the standard for counting naevi based on images. Results for total numbers of naevi counted on the back yielded substantial agreement and by examining a large number of participants the effect of non-differential misclassifications was likely levelled off. Misclassifications related to size estimation of naevi were observed both between methods (manual versus remote evaluation) and in-between raters, even if agreement was still moderate to substantial for naevi ≥ 2 mm. As naevi in children often borders 2 mm, there will always be uncertainty in the size-estimation process that may risk undermining the temporal benefits of using mobile teledermatology, thus we propose that counting all naevi will offer a more feasible approach.

In addition to validating mobile teledermatology for the ability to correctly assess number of naevi, several practical observations were also attained. For example, the illumination in the examining rooms was only from fluorescent lights in the ceiling and additional natural light from the window, naturally shifting depending on weather, season and time of day. The static

positioning of the hand-held mobile camera generated oblique projections of naevi located on the flanking parts of the back which to some extent obscured estimating of naevi size in these areas.

It was thus perceived that the digital imaging would result in a higher proportion of low quality images. Notwithstanding, the majority of the digital images, 74% and 64% respectively, were noted as "fairly good" and none had to be discarded due to too poor quality. The results suggest that the mobile camera used in this study holds a sufficient and robust technology which can substantiate its potential use also in non-hospital settings. However, a non-negligible proportion of the images were not transmitted through the mobile teledermatology system and any one reason for this could not be clarified. A routine for technical assistance needs to be established if aiming to develop the method for more large-scale use.

A valid measure for the time consumption in manual and remote assessment of naevi prevalence was not fully practicable in the study. Especially as the manual counting and digital imaging was integrated in the scheduled doctors' visit. However, by rough calculation the procedure of the manual counting and recording of skin photo type, hair and eye colour on the paper chart varied between 5-7 minutes per child.
The amount of time for logging in on the mobile application Dermicus and performing in total four images took on average 3 minutes per child.
The counting, size estimations and recordings of naevi on the paper chart by the two dermatologists viewing the digital images took approximately 5 minutes per child.

In an additional analysis an "intra-rater/ inter-method reliability" analysis was performed by the dermatologist performing the manual counting. Even if the counting of naevi from the digital images was performed 2 months after having completed the face-to-face manual counting of naevi, this analysis was thought to bring a small, but not negligible, risk of recall bias and was not included in the manuscript. Interestingly, the results did show comparable, but not superior, results when compared with the two dermatologists performing the counting of naevi exclusively from images.

STUDY IV

In Study IV, both the naevi studies in children and melanoma data in adults were population-based. A major advantage of using population-based data (if receiving high participation rates) is that these will be representative of the populations' variety of genetic factors associated with naevi and melanoma proneness, interacting with the ambient UV and life styles factors related to sun exposure practices. However, it is not possible to weight the impact of genetic and environmental factors separately as the basic genetic prerequisites for "moliness" and melanoma risk in the northern and southern populations were not known.

The pigmentary phenotypes assessed in the children residing in northern and southern Sweden in 2001/2002 were homogenous regarding hair colour and basic skin photo types (predominantly blond and blue/grey-eyed) in both northern and southern Sweden but more children in the north were brown-eyed. Data extracted from Statistics Sweden demonstrated that the number of inhabitants with a foreign ancestry, presumably of a darker pigmentation, was lower in the north. More children with brown eyes might be related to the Sami population, being indigenous in northern Sweden and especially in Kiruna. A genome-wide single nucleotide polymorphism study by Salmela et al. has demonstrated genetic variations in-between populations in different geographic regions in Sweden (228). Whether this has implications on naevi prevalence is not known but incidence of melanoma

is lower in Sami men, however not in Sami women (229) compared with the Swedish population at large.

When assessing risk of melanoma per body site, many researchers have used the relative melanoma numbers or incidences per body site as an arbitrary measure of melanoma density (230-232). Some have even adjusted for differences in melanocyte density in different body sites (233). In Study IV we decided to compare the anatomic distributions of melanoma and childhood naevi using the crude numbers as relative proportions on the face, upper extremities, the trunk and lower extremities. The individual height and weight in the melanoma patients was not known and a corresponding calculation of regional BSAs as done with naevi densities in the children could therefore not be performed. Hence, it was not possible to adjust for the differences in specific body-site proportions between adults and children or between males and females. However, when considering naevi as risk markers for melanoma there is evidence that body size is not a variable of significance and larger individuals do not display more naevi (234). The present state of knowledge is that both the number and density of naevi should be considered equally valid as markers of the risk of melanoma.

8 CONCLUSIONS

STUDY I

Study I gave indications of increased parental awareness regarding the risks of sun overexposure in recent years, even though travelling to sunny holiday resorts abroad had become more frequent.

The parents in 2007 reported overall more frequent use of various sun protective regimens and, in line with general recommendations, especially regarding physical measures (clothing, shade, staying indoors).

The results of this study acknowledged the close relationship between changes in sun-protective behaviour and the subsequent effect on naevi prevalence in children. The results supported a model use of childhood naevi as an objective indicator of UV exposure and as a potential tool for studying trends in sun exposure in children.

STUDY II

Study II demonstrated that mobile teledermatology is an easy applicable method, providing clinical images of children valid for remote counting of the total number of naevi on the back.

The technique needs to be further investigated in non-hospital settings, e.g. by involving medical personnel in health centres or schools. Implemented on a broader population basis mobile teledermatology has the potential to facilitate the surveillance of trends in sun exposure among children.

STUDY III

In Study III a shift in the anatomical naevi distribution pattern between 2002 and 2007 among 7-year-old children in southern Sweden was demonstrated.

The overall lower total naevi densities registered in 2007 were mainly due to a reduction of naevi on intermittently sun exposed sites like the trunk and extremities. Naevi densities on chronically sun exposed body sites such as the face and dorsal of hands, and rarely sun exposed sites had remained unaltered. The largest reduction in total and site specific numbers of naevi was seen among boys.

STUDY IV

In Study IV a consistency in the body-site distributions and gender profiles of melanoma in young adults and naevi in children was demonstrated. Along with overall significantly higher numbers, a slightly larger proportion of naevi and melanoma were located on the trunk in southern Sweden. More of the clearly sun-induced melanoma subtypes, such as SSM and LMM, were found in southern Sweden.

Results supported that ambient sun exposure is a major determinant of melanocyte proliferation and may provide a scientific basis for better targeted sun protective regimens in children depending on latitude of residing.

9 IMPLICATIONS AND FUTURE PERSPECTIVES

The four studies presented in this thesis were performed with the ultimate objective to increase our knowledge on naevi prevalence and body-site localization in Swedish children in relation to population trends in sun exposure and sun protection. Along the road, several learning points were achieved and questions for potential implications in the future were raised.

Overall, the results in study I and III substantiated the model use of childhood naevi as an objective population indicator of UV exposure. The methodology of counting naevi can suggestively be used for iterated monitoring of naevi prevalence in age-standardized populations of children with the objective to follow current trends. Childhood naevi can also be suitable as biomarkers if performing interventional trials set to improve sun protection. The knowledge regarding the time frame for acquiring new naevi after critical doses of sun exposure in relation to genetically driven naevogenesis is still an issue that lacks substantial understanding.

Internet-based health programs are likely to be more used in routine medical surveillance in the future. The conclusions of Study II were that mobile teledermatology has to be investigated in "the field" to fully assess the potential use for monitoring naevi prevalence in children. If mobile teledermatology was to become an integrated part of a routine school nurse examination, this could potentially provide a feasible approach. Ultimately, an automatized system for identifying naevi, integrated with the mobile application may be a potential task for the future. The safe-coding of the login system and secure storage of the images will be of principal importance.

The learning points from Study IV regarding the impact of early life sun exposure practices and the subsequent risks of melanoma would ideally be implemented by more vigilant protection of children in southern Sweden. Interestingly, the results from Study III indicated that this is already in the making with the findings of lower naevi densities on intermittently sun exposed body sites, like trunk and limbs among children in southern Sweden. The results are interesting in that melanoma incidence in recent decades has increased mainly on intermittently sun exposed body sites. It will thereby be of interest to evaluate if the selective lowering of childhood naevi on intermittently sun exposed body sites in southern Sweden will translate to a future reduction of melanoma on these same sites.

The improvements in sun exposures and sun protection among children in southern Sweden that were demonstrated in these studies are in line with recent national and international reports. Results are encouraging, and underscore the importance of pursuing primary prevention campaigns. Children who grow up more accustomed to the use of various sun protective measures will hopefully bring these routines onward into adult life and to the next generation. However, trends in sun tanning and sun protection come and go and intensifying campaigns targeting adolescents and young adults are other initiatives that hold a challenge for the future. Also, finding ways to promote natural sun shade for children, not focusing too much on sunscreens should be encouraged. For example by providing environments that stimulate outdoor activities while offering moderate shade, e.g. at preschools, schools or when children perform sports or visit sunny holiday resorts.

10 SUMMARY IN SWEDISH/POPULÄRVETENSKAPLIG SAMMANFATTNING

Primärprevention är en viktig hörnsten i statens miljömålsarbete för att minska förekomsten av hudmelanom (melanom) i Sverige. Insjuknandet i melanom stiger med cirka 5 % per år vilket tros vara relaterat till ett riskfyllt solskyddsbeteende och ett ökat antal solresor utomlands. Utvecklingen av vanliga, förvärvade födelsemärken, s.k. pigmentnaevi (naevi), är nära kopplat till graden av solexposition. Stark solexposition, särskilt tidigt i livet ökar risken att utveckla många naevi och påverkar individens framtida risk att få melanom.

Studie I var en populationsbaserad tvärsnittsstudie som genomfördes 2002 och 2007 i kommunerna Ljungby och Falkenberg i södra Sverige. Båda åren undersöktes alla 7-åriga barn, deras naevi räknades och uppgift om ögonfärg/hårfärg/hudtyp registrerades. Föräldrarna besvarade samtidigt en enkät gällande barnets solresor, antal solbrännor och användandet av solskydd. Resultat av föräldraenkäten visade på signifikant förbättringar av solskyddande åtgärder (solkräm, klädsel, söka skugga) 2007 jämfört med 2002. Trots att antalet solresor hade ökat 2007 var medelantalet naevi hos barnen signifikant lägre. Resultaten visade att föräldrar i Sverige på senare år solskyddar sina barn bättre samt att naevi hos barn kan användas som ett objektivt populationsmått på solexponering.

I **Studie II** var syftet att validera om räkning av naevi från digitala foton som tagits via en mobiltelefon och en nedladdad ”app”, s.k. mobil teledermatologi, var jämförbar med traditionell, manuell räkning av naevi. Nittiosju barn undersöktes med den manuella metoden och digitala foton togs av barnets rygg med hjälp av en mobilkamera där bilderna skickades via en CE-märkt ”app”; Dermicus. Två oberoende dermatologer räknade naevi enbart utifrån de digitala fotona. Resultaten visade på mycket god överensstämmelse mellan metoderna (inter-method reliability) samt mellan undersökare (inter-rater reliability). Mobil teledermatologi visades vara en valid metod för att indirekt räkna naevi på ryggen hos barn vilket öppnar för en möjlig användning av metoden för att följa trender i solexponering/solskydd.

I **Studie III** undersöktes om den signifikanta minskning av naevi som skett hos 7-åriga barn mellan 2002 och 2007 i södra Sverige (Studie I) skett i samma utsträckning på alla kroppsytor och om den varit lika stor mellan könen. Antalet naevi på 16 olika kroppsytor hos 1189 barn (595 pojkar och 594 flickor) fanns registrerade. 2007 hade en signifikant minskning av naevi skett på intermittent belysta kroppsytor, såsom bål och extremiteter medan ingen förändring sågs på kroniskt och sällan solexponerade ytor. Pojkar hade i genomsnitt 5 färre naevi och flickor i genomsnitt 3 färre naevi 2007 jämfört med 2002. Denna minskning av naevi på intermittent belysta kroppsytor talar för en effektiv tillämpning av solskydd hos barn på senare år och kan förhoppningsvis förespå en framtida minskning av melanom på dessa kroppsytor.

I **Studie IV** var syftet att studera skillnader i melanominsjuknande och melanomtyper mellan norra och södra Sverige samt undersöka om den anatomiska distributionen av melanom överensstämde med distributionen av naevi hos barn boende i samma geografiska regioner. Resultaten visade att melanomincidensen i södra Sverige är dubbelt så hög mot i norr och 7-åriga barn boende i södra Sverige har motsvarande cirka 80 % högre naevidensitet. Fördelningen av naevi på olika kroppsytor (ansikte, övre extremiteter, bål, nedre extremiteter) hos barn i norra respektive södra Sverige motsvarande bäst fördelning av melanom hos yngre och medelålders vuxna. Något fler melanom respektive naevi var lokaliserade på bålen i södra Sverige. Gemensamma könsprofiler mellan melanom och naevi påvisades: män/pojkar hade flest melanom/naevi på bålen medan kvinnor/flickor hade fler melanom/naevi på benen. Resultaten bekräftar att både antalet och fördelningen av naevi hos barn speglar risken för melanom samt att solexposition tidigt i livet utgör en viktig miljöfaktor.

11 ACKNOWLEDGEMENTS

Ylva Rodvall, my main supervisor. My deepest thanks for taking me on as a graduate student and for introducing me to the field of public health research. You have given me invaluable insights to the complex task of conducting good epidemiological research. Not at least that a network of committed collaborators is essential for obtaining high quality data when performing clinical studies. Your devotion to this project and your honest belief that sun protection in young children is of critical importance is truly inspirational.

Carl-Fredrik Wahlgren, my co-supervisor. You hold all the good virtues in life, being a super-duper supervisor, researcher, clinician and teacher as well as being the most dedicated grandfather. Thank you for always being so considerate, boosting my self-confidence when I need it the most and thank you for bringing me to eat proper warm lunch when I tend to stick with a cold salad.

Kerstin Wiklund, my co-supervisor. With your kind and humble appearance coupled with a mind so immensely sharp and excellent you have guided me through this thesis. To lean on your expertise when it comes to implementing statistics based on the art of the data is the real meaning of power. Thank you!

Bernt Lindelöf, my co-supervisor. Thank you for being part of my doctoral years, contributing with your genuine knowledge in skin cancer epidemiology. Always open-minded, up-to-date and sharing with us interesting topics during research meetings and lunch breaks. I hope and believe that we will go on discussing research on the bus to and from Tyresö.

Yvonne Höijer, research nurse in all naevi studies. Your warmth and professionalism in collaboration with the school personnel and in contact with the children has been a joy to be part of. The impressive work you have performed (counting more than 50.000 naevi!) has been a prerequisite for this research and I want to thank you ever so much.

Ylva Trolle Lagerros, my mentor and former head of the research school for clinicians in epidemiology. Thank you for sharing with me your personal experiences in pursuing research alongside with clinical work, caring for a family and upon that being a skilful horsewoman. I still haven't figured out how you manage it! I much appreciate the way you have "infused" me with tons of good advice during brisk walks around Hagaparken.

Lena Lundeberg, head of the Department of Dermatology and *Toomas Talme,* my closest boss. Thank you for caring so much for your personnel and for providing the best of conditions for combining hard clinical work with research.

Mona Ståhle, head of the Dermatology and Venereology Unit, Department of Medicine, Solna. Thank you for always sharing with us the latest advances within dermatological research. This brings the spark to clinical work that visualises what we can achieve for our patients in the near future.

Gunilla Ekstrand, research secretary at the Dermatology and Venereology Unit. Your cheerful mind and tranquil approach has been of invaluable help along the sometimes winding pathways of research administration.

Johan Heilborn, my former colleague and inventor of the Dermicus mobile teledermatology system. Thank you deeply for introducing me to your brilliant invention and letting me use it for research purposes. You have been extremely supportive in every step of the way and ready to assist whenever needed during the course of the project.

To *all my colleagues, nurses and administrative staff at the Department of Dermatology*, for making every day's work inspiring and sharing a true interest in dermatology. Together we can achieve great things for our patients and I feel truly proud to be a part of this "dream team". I would like to especially thank:

Jan Lapins, the most reputable clinician and tutor in the art of handling skin tumours. Thank you for valuable discussions at all times, even when you are in the middle of elk hunting. Your enthusiasm for the latest research publications, sharing them with all your colleagues is so inspiring and your belief in my research and in this thesis has contributed immensely.

Maria Böhme, good friend and colleague, you exert the leadership at the Paediatric dermatology clinic with such friendliness and gentle firmness. Your sensibility and skills in the art of paediatric dermatology is all what I hope to achieve in my professional life and also I hope to eventually learn to adopt your sense for really good coffee.

Natalia Kuzmina, my roommate and good friend. You are such a dedicated doctor, always exploring the edges of new science, all in the best interest of your patients. Your warm personality makes me happy every time I open the door to our room. Thank you for inspiring me and my family to visit cultural events and exciting destinations (hitherto only in my fantasy, but soon this will change!).

Hanna Eriksson, my friend and former colleague. I am greatly impressed with your stamina and determination to become an oncologist as well as dermatologist. Melanoma research and the patients will thank you for your strong commitment and I hope we can go on working and lecturing together in the future.

Josefin Lysell, thank you for being such a good friend and thank you for sharing with me all your knowledge within the field of childhood psoriasis and beyond. I wish you the best of luck in your forthcoming dissertation!

Desiree Wiegleb Edström, your clinical and tutorial skills, your never-ending enthusiasm and lack of prestige makes you the best colleague ever to hang out with at work; and not to say after work! Attending to your running practice in Hagaparken is my next goal in life.

The nurses at the Pediatric dermatology clinic, *Ann-Charlotte, Britt-Marie, Madeleine and Caroline.* You provide great care for the children and parents and hold such genuine experience. Thank you especially for helping out with the mobile teledermatology study and for showing such an interest in my research throughout these years.

The nurses at Skin Treatment Center, *Annika, Lena, Leena, Josefin, Noomi, Nina and Anders.* Thank you for being such excellent people and co-workers. Working together has always been a joy, bringing many laughs along the way.

All former and present members of the Journal Club, *Carl-Fredrik Wahlgren, Maria Böhme, Maria Bradley, Desiree Wiegleb Edström, Emma Johansson, Natalia Ballardini and Maria Lagrelius.* Thank you for wonderful discussions and laughter's while dissecting new, old or simply quite odd research articles.

Britta Krynitz, my friend and former colleague back in the days when we were resident physicians at the Department of Dermatology. Thank you for kindly letting me share your supervisor Bernt with you. I admire your hard work and look forward to attending your soon to come dissertation!

All my friends at the research school for clinicians in epidemiology, especially *Sigga Björnsdottir and Carina Grönhagen.* Thank you for being so friendly, helpful and enthusiastic. You possess skills in epidemiology that will lead you far and I hope we can initiate research projects together in the future.

My mother *Barbro* and my father *Christer*, you provide unconditional love and support, taking care of me and my family at all times and you host a top star day-care for our dog. I also wish to thank my mother-in-law *Dagmar*, for being such a wonderful and sporty super-granny. To my late grandmother *Anna-Lisa*, you are with me every step of the way…

My brother *Daniel* and sister-in-law *Anneli* and daughter *Désirée*, the best people ever. Thank you for letting me and my family take part of your culinary excesses and for teaching us what is most important in life; music, culture, food… and football.

Pernilla Hugoson, my cousin and sister at heart and her fantastic family *Erik* and *Hannes*. I treasure our friendship more than words can say. I am so glad that you have started up PhD studies in music therapy in neonates and I know you will do brilliantly. I look forward to us taking that walk around the coast of Fårö when life settles down a bit!

To all good friends, especially the Otterström family, *Lena, Patrick, Alexandra and Douglas.* Thank you for all the vivid discussions, games, laughs and the wonderful trips we have spent together and the many, many more to come. The Hedin family, *Cecilia, Gunnar, Linnea, Arvid and Mattias* and the Ewald family, *Louise, Leif, Elin and Max.* Thank you for being such great and inspiring friends and neighbours.

To my oldest and dearest friends *Marie and Yvonne*, thank you for always being there.

All my wonderful cousins, uncles and aunties, *Göran, Helen, Peppe, Sanna, Milla, Andreas, Sofia, Tony, Vilmer, Mirja, Gunnar, Sandra, Jimmie, Catherine, Kacie.* Knowing that you will always stand by me no matter what makes life great. And thanks to my fantastic family-in-law *Jan-Erik, Eva, Johan, Tina, Signe, Sture, Ulrika, Ann-Christin, Evald, Fredrik, Rebecka, Hanna and Johan.*

My husband *Claes*, my companion for life and the most committed sportsman there ever was, always exploring new ways of practising your excess energy. The love and trust we share is the foundation for all things great and small!

My three children *Sara, Erik and Max,* you are the true miracles in life. Watching you grow up to become such fantastic individuals; good-hearted, strong and bright has been the most amazing experience. I love you more than life. And to my "son-in-law" *Victor,* thank you for bringing positive energy and so much laughter to our family.

12 REFERENCES

1. Sybert VP. Development of melanocytic nevi in children. Arch Dermatol 1997; 133: 1049
2. Gallagher RP, McLean DI, Yang CP, Coldman AJ, Silver HK, Spinelli JJ, et al. Suntan, sunburn, and pigmentation factors and the frequency of acquired melanocytic nevi in children. Similarities to melanoma: the Vancouver Mole Study. Arch Dermatol 1990; 126: 770-776
3. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 2005; 41: 45-60
4. Holman CD, Armstrong BK. Pigmentary traits, ethnic origin, benign nevi, and family history as risk factors for cutaneous malignant melanoma. J Natl Cancer Inst 1984; 72: 257-266
5. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Abeni D, Boyle P, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer 2005; 41: 28-44
6. Bauer J, Garbe C. Acquired melanocytic nevi as risk factor for melanoma development. A comprehensive review of epidemiological data. Pigment Cell Research 2003; 16: 297-306
7. Skender-Kalnenas TM, English DR, Heenan PJ. Benign melanocytic lesions: risk markers or precursors of cutaneous melanoma? J Am Acad Dermatol 1995; 33: 1000-1007
8. Pfahlberg A, Uter W, Kraus C, Wienecke WR, Reulbach U, Kolmel KF, et al. Monitoring of nevus density in children as a method to detect shifts in melanoma risk in the population. Prev Med 2004; 38: 382-387
9. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Zanetti R, Masini C, et al. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer 2005; 41: 2040-2059
10. English DR, Milne E, Simpson JA. Ultraviolet radiation at places of residence and the development of melanocytic nevi in children (Australia). Cancer Causes Control 2006; 17: 103-107
11. Green A, Sorahan T, Pope D, Siskind V, Hansen M, Hanson L, et al. Moles in Australian and British school children. Lancet 1988; 2: 1497
12. Harrison SL, MacKie RM, MacLennan R. Development of melanocytic nevi in the first three years of life. J Natl Cancer Inst 2000; 92: 1436-1438
13. Karlsson P, Stenberg B, Rosdahl I. Prevalence of pigmented naevi in a Swedish population living close to the Arctic Circle. Acta Derm Venereol 2000; 80: 335-339
14. Bishop JA, Bradburn M, Bergman W, Osterlind A, Pinney E, Rosdahl I, et al. Teaching non-specialist health care professionals how to identify the atypical mole syndrome phenotype: a multinational study. Br J Dermatol 2000; 142: 331-337
15. Augustsson A, Stierner U, Suurkula M, Rosdahl I. Prevalence of common and dysplastic naevi in a Swedish population. Br J Dermatol 1991; 124: 152-156
16. Rodvall Y, Wahlgren CF, Ullen H, Wiklund K. Common melanocytic nevi in 7-year-old schoolchildren residing at different latitudes in Sweden. Cancer Epidemiol Biomarkers Prev 2007; 16: 122-127
17. Rawles ME. Origin of pigment cells from the neural crest in the mouse embryo. Physiol Zool 1947; 20: 248-266

18. Yamaguchi Y, Morita A, Maeda A, Hearing VJ. Regulation of skin pigmentation and thickness by Dickkopf 1 (DKK1). J Investig Dermatol Symp Proc 2009; 14: 73-75
19. Scott G. Demonstration of melanosome transfer by a shedding microvesicle mechanism. J Invest Dermatol 2012; 132: 1073-1074
20. Szabo G. The Regional Anatomy of the Human Integument with Special Reference to the Distribution of Hair Follicles, Sweat Glands and Melanocytes. Phil Trans R Soc Lond B 1967; 252: 447-485
21. Oetting WS, Brilliant MH, King RA. The clinical spectrum of albinism in humans. Mol Med Today 1996; 2: 330-335
22. Yamaguchi Y, Brenner M, Hearing VJ. The regulation of skin pigmentation. J Biol Chem 2007; 282: 27557-27561
23. Langan EA, Nie Z, Rhodes LE. Melanotropic peptides: more than just 'Barbie drugs' and 'sun-tan jabs'? Br J Dermatol 2010; 163: 451-455
24. Burian E, Burian E. [Eruptive nevi after injection of drugs marketed as melanotan II. The first two Swedish cases described]. Lakartidningen 2013; 110: 208-210
25. Conroy JD. Melanocytic tumors of domestic animals with special reference to dogs. Arch Dermatol 1967; 96: 372-380
26. Bauer J, Buttner P, Wiecker TS, Luther H, Garbe C. Risk factors of incident melanocytic nevi: a longitudinal study in a cohort of 1,232 young German children. Int J Cancer 2005; 115: 121-126
27. Oliveria SA, Scope A, Satagopan JM, Geller AC, Dusza SW, Weinstock MA, et al. Factors associated with nevus volatility in early adolescence. J Invest Dermatol 2014; 134: 2469-2471
28. Oliveria SA, Yagerman SE, Jaimes N, Goodwin AI, Dusza SW, Halpern AC, et al. Clinical and dermoscopic characteristics of new naevi in adults: results from a cohort study. Br J Dermatol 2013; 169: 848-853
29. Sommer L. Generation of melanocytes from neural crest cells. Pigment Cell Melanoma Res 2011; 24: 411-421
30. Scope A, Marghoob AA, Chen CS, Lieb JA, Weinstock MA, Halpern AC, et al. Dermoscopic patterns and subclinical melanocytic nests in normal-appearing skin. Br J Dermatol 2009; 160: 1318-1321
31. Bastian BC. The molecular pathology of melanoma: an integrated taxonomy of melanocytic neoplasia. Annu Rev Pathol 2014; 9: 239-271
32. Piliouras P, Gilmore S, Wurm EM, Soyer HP, Zalaudek I. New insights in naevogenesis: number, distribution and dermoscopic patterns of naevi in the elderly. Australas J Dermatol 2011; 52: 254-258
33. Zalaudek I, Schmid K, Marghoob AA, Scope A, Manzo M, Moscarella E, et al. Frequency of dermoscopic nevus subtypes by age and body site: a cross-sectional study. Arch Dermatol 2011; 147: 663-670
34. Argenziano G, Zalaudek I, Ferrara G, Hofmann-Wellenhof R, Soyer HP. Proposal of a new classification system for melanocytic naevi. Br J Dermatol 2007; 157: 217-227
35. Scope A, Dusza SW, Marghoob AA, Satagopan JM, Braga Casagrande Tavoloni J, Psaty EL, et al. Clinical and dermoscopic stability and volatility of melanocytic nevi in a population-based cohort of children in Framingham school system. J Invest Dermatol 2011; 131: 1615-1621
36. Scope A, Marghoob AA, Dusza SW, Satagopan JM, Agero AL, Benvenuto-Andrade C, et al. Dermoscopic patterns of naevi in fifth grade children of the Framingham school system. Br J Dermatol 2008; 158: 1041-1049

37. Crombie IK. Racial differences in melanoma incidence. Br J Cancer 1979; 40: 185-193
38. Garbe C, Leiter U. Melanoma epidemiology and trends. Clin Dermatol 2009; 27: 3-9
39. Lancaster HO. Some geographical aspects of the mortality from melanoma in Europeans. Med J Aust 1956; 43: 1082-1087
40. Khlat M, Vail A, Parkin M, Green A. Mortality from melanoma in migrants to Australia: variation by age at arrival and duration of stay. Am J Epidemiol 1992; 135: 1103-1113
41. Cooke KR, Fraser J. Migration and death from malignant melanoma. Int J Cancer 1985; 36: 175-178
42. Holman CD, Armstrong BK, Heenan PJ, Blackwell JB, Cumming FJ, English DR, et al. The causes of malignant melanoma: results from the West Australian Lions Melanoma Research Project. Recent Results Cancer Res 1986; 102: 18-37
43. Oliveria SA, Saraiya M, Geller AC, Heneghan MK, Jorgensen C. Sun exposure and risk of melanoma. Arch Dis Child 2006; 91: 131-138
44. Eide MJ, Weinstock MA. Association of UV index, latitude, and melanoma incidence in nonwhite populations--US Surveillance, Epidemiology, and End Results (SEER) Program, 1992 to 2001. Arch Dermatol 2005; 141: 477-481
45. Forsea AM, Del Marmol V, de Vries E, Bailey EE, Geller AC. Melanoma incidence and mortality in Europe: new estimates, persistent disparities. Br J Dermatol 2012; 167: 1124-1130
46. Karlsson PM, Fredrikson M. Cutaneous malignant melanoma in children and adolescents in Sweden, 1993-2002: the increasing trend is broken. Int J Cancer 2007; 121: 323-328
47. Erdmann F, Lortet-Tieulent J, Schuz J, Zeeb H, Greinert R, Breitbart EW, et al. International trends in the incidence of malignant melanoma 1953-2008--are recent generations at higher or lower risk? Int J Cancer 2013; 132: 385-400
48. de Vries E, Bray FI, Coebergh JW, Parkin DM. Changing epidemiology of malignant cutaneous melanoma in Europe 1953-1997: rising trends in incidence and mortality but recent stabilizations in western Europe and decreases in Scandinavia. Int J Cancer 2003; 107: 119-126
49. Youl PH, Youlden DR, Baade PD. Changes in the site distribution of common melanoma subtypes in Queensland, Australia over time: implications for public health campaigns. Br J Dermatol 2013; 168: 136-144
50. Iannacone MR, Youlden DR, Baade PD, Aitken JF, Green AC. Melanoma incidence trends and survival in adolescents and young adults in Queensland, Australia. Int J Cancer 2014:
51. Lyth J, Eriksson H, Hansson J, Ingvar C, Jansson M, Lapins J, et al. Trends in cutaneous malignant melanoma in Sweden 1997-2011: Thinner tumours and improved survival among men. Br J Dermatol 2014:
52. National Board of Health and Welfare's Cancer Register 2014. Available from: http://www.socialstyrelsen.se/statistics/statisticaldatabase/cancer.
53. Karlsson P, Boeryd B, Sander B, Westermark P, Rosdahl I. Increasing incidence of cutaneous malignant melanoma in children and adolescents 12-19 years of age in Sweden 1973-92. Acta Derm Venereol 1998; 78: 289-292
54. Baade PD, Green AC, Smithers BM, Aitken JF. Trends in melanoma incidence among children: possible influence of sun-protection programs. Expert Rev Anticancer Ther 2011; 11: 661-664

55. Berg P, Lindelof B. Differences in malignant melanoma between children and adolescents. A 35-year epidemiological study. Arch Dermatol 1997; 133: 295-297
56. Youl P, Aitken J, Hayward N, Hogg D, Liu L, Lassam N, et al. Melanoma in adolescents: a case-control study of risk factors in Queensland, Australia. Int J Cancer 2002; 98: 92-98
57. Lu C, Zhang J, Nagahawatte P, Easton J, Lee S, Liu Z, et al. The Genomic Landscape of Childhood and Adolescent Melanoma. J Invest Dermatol 2014:
58. Cordoro KM, Gupta D, Frieden IJ, McCalmont T, Kashani-Sabet M. Pediatric melanoma: results of a large cohort study and proposal for modified ABCD detection criteria for children. J Am Acad Dermatol 2013; 68: 913-925
59. Whiteman DC, Watt P, Purdie DM, Hughes MC, Hayward NK, Green AC. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. J Natl Cancer Inst 2003; 95: 806-812
60. Lee JH, Choi JW, Kim YS. Frequencies of BRAF and NRAS mutations are different in histological types and sites of origin of cutaneous melanoma: a meta-analysis. Br J Dermatol 2011; 164: 776-784
61. Chang YM, Newton-Bishop JA, Bishop DT, Armstrong BK, Bataille V, Bergman W, et al. A pooled analysis of melanocytic nevus phenotype and the risk of cutaneous melanoma at different latitudes. Int J Cancer 2009; 124: 420-428
62. Parra EJ. Human pigmentation variation: evolution, genetic basis, and implications for public health. Am J Phys Anthropol 2007; Suppl 45: 85-105
63. Juzeniene A, Setlow R, Porojnicu A, Steindal AH, Moan J. Development of different human skin colors: a review highlighting photobiological and photobiophysical aspects. J Photochem Photobiol B 2009; 96: 93-100
64. Man MQ, Lin TK, Santiago JL, Celli A, Zhong L, Huang ZM, et al. Basis for enhanced barrier function of pigmented skin. J Invest Dermatol 2014; 134: 2399-2407
65. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 1988; 124: 869-871
66. Falk M. Differences in sun exposure habits between self-reported skin type and ultraviolet sensitivity measured by phototest. Photodermatol Photoimmunol Photomed 2011; 27: 190-195
67. Falk M. Self-estimation or phototest measurement of skin UV sensitivity and its association with people's attitudes towards sun exposure. Anticancer Res 2014; 34: 797-803
68. Oliveria SA, Satagopan JM, Geller AC, Dusza SW, Weinstock MA, Berwick M, et al. Study of Nevi in Children (SONIC): baseline findings and predictors of nevus count. Am J Epidemiol 2009; 169: 41-53
69. Dellavalle RP, Johnson KR, Hester EJ, Deas AM, Mokrohisky S, Morelli JG, et al. Children with red hair have more freckles but fewer melanocytic nevi: results from a cohort study of 280 three-year-olds. Arch Dermatol 2005; 141: 1042-1043
70. Darlington S, Siskind V, Green L, Green A. Longitudinal study of melanocytic nevi in adolescents. J Am Acad Dermatol 2002; 46: 715-722
71. Valverde P, Healy E, Jackson I, Rees JL, Thody AJ. Variants of the melanocyte-stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nat Genet 1995; 11: 328-330
72. Reimer RR, Clark WH, Jr., Greene MH, Ainsworth AM, Fraumeni JF, Jr. Precursor lesions in familial melanoma. A new genetic preneoplastic syndrome. JAMA 1978; 239: 744-746

73. Tucker MA, Halpern A, Holly EA, Hartge P, Elder DE, Sagebiel RW, et al. Clinically recognized dysplastic nevi. A central risk factor for cutaneous melanoma. JAMA 1997; 277: 1439-1444
74. Vredenborg A, Bohringer S, Boonk SE, Gruis NA, Out-Luijting C, Kukutsch NA, et al. Acquired melanocytic nevi in childhood and familial melanoma. JAMA Dermatol 2014; 150: 35-40
75. Augustsson A. Melanocytic naevi, melanoma and sun exposure. Acta Derm Venereol Suppl (Stockh) 1991; 166: 1-34
76. Autier P, Boniol M, Severi G, Pedeux R, Grivegnee AR, Dore JF. Sex differences in numbers of nevi on body sites of young European children: implications for the etiology of cutaneous melanoma. Cancer Epidemiology, Biomarkers & Prevention 2004; 13: 2003-2005
77. Gallagher RP, McLean DI, Yang CP, Coldman AJ, Silver HK, Spinelli JJ, et al. Anatomic distribution of acquired melanocytic nevi in white children. A comparison with melanoma: the Vancouver Mole Study. Arch Dermatol 1990; 126: 466-471
78. Kwan TY, Belke TW, Enta T. Sex differences in the anatomical distribution of melanocytic nevi in Canadian Hutterite children. J Cutan Med Surg 2000; 4: 58-62
79. Baron AE, Asdigian NL, Gonzalez V, Aalborg J, Terzian T, Stiegmann RA, et al. Interactions between Ultraviolet Light and MC1R and OCA2 Variants Are Determinants of Childhood Nevus and Freckle Phenotypes. Cancer Epidemiol Biomarkers Prev 2014:
80. Bataille V, Snieder H, MacGregor AJ, Sasieni P, Spector TD. Genetics of risk factors for melanoma: an adult twin study of nevi and freckles. J Natl Cancer Inst 2000; 92: 457-463
81. Zhu G, Duffy DL, Eldridge A, Grace M, Mayne C, O'Gorman L, et al. A major quantitative-trait locus for mole density is linked to the familial melanoma gene CDKN2A: a maximum-likelihood combined linkage and association analysis in twins and their sibs. Am J Hum Genet 1999; 65: 483-492
82. Wachsmuth RC, Turner F, Barrett JH, Gaut R, Randerson-Moor JA, Bishop DT, et al. The effect of sun exposure in determining nevus density in UK adolescent twins. J Invest Dermatol 2005; 124: 56-62
83. Easton DF, Cox GM, Macdonald AM, Ponder BA. Genetic susceptibility to naevi--a twin study. Br J Cancer 1991; 64: 1164-1167
84. Hussussian CJ, Struewing JP, Goldstein AM, Higgins PA, Ally DS, Sheahan MD, et al. Germline p16 mutations in familial melanoma. Nat Genet 1994; 8: 15-21
85. Hayward NK. Genetics of melanoma predisposition. Oncogene 2003; 22: 3053-3062
86. Helgadottir H, Hoiom V, Jonsson G, Tuominen R, Ingvar C, Borg A, et al. High risk of tobacco-related cancers in CDKN2A mutation-positive melanoma families. J Med Genet 2014; 51: 545-552
87. Hill VK, Gartner JJ, Samuels Y, Goldstein AM. The genetics of melanoma: recent advances. Annu Rev Genomics Hum Genet 2013; 14: 257-279
88. Norton HL, Kittles RA, Parra E, McKeigue P, Mao X, Cheng K, et al. Genetic evidence for the convergent evolution of light skin in Europeans and East Asians. Mol Biol Evol 2007; 24: 710-722
89. Bishop DT, Demenais F, Iles MM, Harland M, Taylor JC, Corda E, et al. Genome-wide association study identifies three loci associated with melanoma risk. Nat Genet 2009; 41: 920-925

90. Fallah M, Pukkala E, Sundquist K, Tretli S, Olsen JH, Tryggvadottir L, et al. Familial melanoma by histology and age: joint data from five Nordic countries. Eur J Cancer 2014; 50: 1176-1183
91. Berg P, Wennberg AM, Tuominen R, Sander B, Rozell BL, Platz A, et al. Germline CDKN2A mutations are rare in child and adolescent cutaneous melanoma. Melanoma Res 2004; 14: 251-255
92. Albino AP, Le Strange R, Oliff AI, Furth ME, Old LJ. Transforming ras genes from human melanoma: a manifestation of tumour heterogeneity? Nature 1984; 308: 69-72
93. Bauer J, Curtin JA, Pinkel D, Bastian BC. Congenital melanocytic nevi frequently harbor NRAS mutations but no BRAF mutations. J Invest Dermatol 2007; 127: 179-182
94. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature 2002; 417: 949-954
95. Broekaert SM, Roy R, Okamoto I, van den Oord J, Bauer J, Garbe C, et al. Genetic and morphologic features for melanoma classification. Pigment Cell Melanoma Res 2010; 23: 763-770
96. Edlundh-Rose E, Egyhazi S, Omholt K, Mansson-Brahme E, Platz A, Hansson J, et al. NRAS and BRAF mutations in melanoma tumours in relation to clinical characteristics: a study based on mutation screening by pyrosequencing. Melanoma Res 2006; 16: 471-478
97. Maldonado JL, Fridlyand J, Patel H, Jain AN, Busam K, Kageshita T, et al. Determinants of BRAF mutations in primary melanomas. J Natl Cancer Inst 2003; 95: 1878-1890
98. Thomas NE, Edmiston SN, Alexander A, Millikan RC, Groben PA, Hao H, et al. Number of nevi and early-life ambient UV exposure are associated with BRAF-mutant melanoma. Cancer Epidemiol Biomarkers Prev 2007; 16: 991-997
99. Pollock PM, Harper UL, Hansen KS, Yudt LM, Stark M, Robbins CM, et al. High frequency of BRAF mutations in nevi. Nat Genet 2003; 33: 19-20
100. Tschandl P, Berghoff AS, Preusser M, Burgstaller-Muehlbacher S, Pehamberger H, Okamoto I, et al. NRAS and BRAF mutations in melanoma-associated nevi and uninvolved nevi. PLoS One 2013; 8: e69639
101. Chu EY, Wanat KA, Miller CJ, Amaravadi RK, Fecher LA, Brose MS, et al. Diverse cutaneous side effects associated with BRAF inhibitor therapy: a clinicopathologic study. J Am Acad Dermatol 2012; 67: 1265-1272
102. Yagerman S, Flores E, Busam K, Lacouture M, Marghoob AA. Overview photography and short-term mole monitoring in patients taking a BRAF inhibitor. JAMA Dermatol 2014; 150: 1010-1011
103. El Ghissassi F, Baan R, Straif K, Grosse Y, Secretan B, Bouvard V, et al. A review of human carcinogens--part D: radiation. Lancet Oncol 2009; 10: 751-752
104. Soter NA. Acute effects of ultraviolet radiation on the skin. Semin Dermatol 1990; 9: 11-15
105. Spatz A, Giglia-Mari G, Benhamou S, Sarasin A. Association between DNA repair-deficiency and high level of p53 mutations in melanoma of Xeroderma pigmentosum. Cancer Res 2001; 61: 2480-2486
106. Pfeifer GP, Besaratinia A. UV wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer. Photochem Photobiol Sci 2012; 11: 90-97
107. Armstrong BK, Kricker A. How much melanoma is caused by sun exposure? Melanoma Res 1993; 3: 395-401

108. Mouret S, Baudouin C, Charveron M, Favier A, Cadet J, Douki T. Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. Proc Natl Acad Sci U S A 2006; 103: 13765-13770
109. Mitra D, Luo X, Morgan A, Wang J, Hoang MP, Lo J, et al. An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. Nature 2012; 491: 449-453
110. Schneider S, Kramer H. Who uses sunbeds? A systematic literature review of risk groups in developed countries. J Eur Acad Dermatol Venereol 2010; 24: 639-648
111. Westerdahl J, Olsson H, Masback A, Ingvar C, Jonsson N, Brandt L, et al. Use of sunbeds or sunlamps and malignant melanoma in southern Sweden. Am J Epidemiol 1994; 140: 691-699
112. Colantonio S, Bracken MB, Beecker J. The association of indoor tanning and melanoma in adults: systematic review and meta-analysis. J Am Acad Dermatol 2014; 70: 847-857 e841-818
113. Westerdahl J, Ingvar C, Masback A, Jonsson N, Olsson H. Risk of cutaneous malignant melanoma in relation to use of sunbeds: further evidence for UV-A carcinogenicity. Br J Cancer 2000; 82: 1593-1599
114. Boniol M, Autier P, Boyle P, Gandini S. Cutaneous melanoma attributable to sunbed use: systematic review and meta-analysis. BMJ 2012; 345: e4757
115. Abdel-Malek ZA, Kadekaro AL, Swope VB. Stepping up melanocytes to the challenge of UV exposure. Pigment Cell Melanoma Res 2010; 23: 171-186
116. Synnerstad I, Nilsson L, Fredrikson M, Rosdahl I. Fewer melanocytic nevi found in children with active atopic dermatitis than in children without dermatitis. Arch Dermatol 2004; 140: 1471-1475
117. Balato N, Di Costanzo L, Balato A, Patruno C, Scalvenzi M, Ayala F. Psoriasis and melanocytic naevi: does the first confer a protective role against melanocyte progression to naevi? Br J Dermatol 2011; 164: 1262-1270
118. Chen FW, Tseng D, Reddy S, Daud AI, Swetter SM. Involution of Eruptive Melanocytic Nevi on Combination BRAF and MEK Inhibitor Therapy. JAMA Dermatol 2014:
119. Bovenschen HJ, Tjioe M, Vermaat H, de Hoop D, Witteman BM, Janssens RW, et al. Induction of eruptive benign melanocytic naevi by immune suppressive agents, including biologicals. Br J Dermatol 2006; 154: 880-884
120. Zattra E, Fortina AB, Bordignon M, Piaserico S, Alaibac M. Immunosuppression and melanocyte proliferation. Melanoma Res 2009; 19: 63-68
121. Green AC, Wallingford SC, McBride P. Childhood exposure to ultraviolet radiation and harmful skin effects: epidemiological evidence. Prog Biophys Mol Biol; 107: 349-355
122. Gies P, Roy C, Toomey S, MacLennan R, Watson M. Solar UVR exposures of primary school children at three locations in Queensland. Photochem Photobiol 1998; 68: 78-83
123. Rafanelli C. Effect of environmental factors on solar UV measurements. Radiat Prot Dosimetry 2001; 97: 423-428
124. Lester RA, Parisi AV. Spectral ultraviolet albedo of roofing surfaces and human facial exposure. Int J Environ Health Res 2002; 12: 75-81
125. Diffey BL, Gies HP. The confounding influence of sun exposure in melanoma. Lancet 1998; 351: 1101-1102

126. Whiteman DC, Whiteman CA, Green AC. Childhood sun exposure as a risk factor for melanoma: a systematic review of epidemiologic studies. Cancer Causes & Control 2001; 12: 69-82
127. Viros A, Sanchez-Laorden B, Pedersen M, Furney SJ, Rae J, Hogan K, et al. Ultraviolet radiation accelerates BRAF-driven melanomagenesis by targeting TP53. Nature 2014; 511: 478-482
128. Nair HB, Ford A, Dick EJ, Jr., Hill RH, Jr., VandeBerg JL. Modeling sunscreen-mediated melanoma prevention in the laboratory opossum (Monodelphis domestica). Pigment Cell Melanoma Res 2014; 27: 843-845
129. Loomis CA. Development and morphogenesis of the skin. Adv Dermatol 2001; 17: 183-210
130. Garcia AM, McLaren CE, Meyskens FL, Jr. Melanoma: is hair the root of the problem? Pigment Cell Melanoma Res 2011; 24: 110-118
131. Leiter U, Garbe C. Epidemiology of melanoma and nonmelanoma skin cancer--the role of sunlight. Adv Exp Med Biol 2008; 624: 89-103
132. Lee JA, Strickland D. Malignant melanoma: social status and outdoor work. Br J Cancer 1980; 41: 757-763
133. Vuong K, McGeechan K, Armstrong BK, Investigators A, Investigators GEM, Cust AE. Occupational sun exposure and risk of melanoma according to anatomical site. Int J Cancer 2014; 134: 2735-2741
134. Beral V, Robinson N. The relationship of malignant melanoma, basal and squamous skin cancers to indoor and outdoor work. Br J Cancer 1981; 44: 886-891
135. Holman CD, Gibson IM, Stephenson M, Armstrong BK. Ultraviolet irradiation of human body sites in relation to occupation and outdoor activity: field studies using personal UVR dosimeters. Clin Exp Dermatol 1983; 8: 269-277
136. Dodd AT, Morelli J, Mokrohisky ST, Asdigian N, Byers TE, Crane LA. Melanocytic nevi and sun exposure in a cohort of colorado children: anatomic distribution and site-specific sunburn. Cancer Epidemiology, Biomarkers and Prevention 2007; 16: 2136-2143
137. Dennis LK, Vanbeek MJ, Beane Freeman LE, Smith BJ, Dawson DV, Coughlin JA. Sunburns and risk of cutaneous melanoma: does age matter? A comprehensive meta-analysis. Ann Epidemiol 2008; 18: 614-627
138. Westerdahl J, Olsson H, Ingvar C. At what age do sunburn episodes play a crucial role for the development of malignant melanoma. Eur J Cancer 1994; 30A: 1647-1654
139. Bränström R, Kristjansson S, Dal H, Rodvall Y. Sun exposure and sunburn among Swedish toddlers. Eur J Cancer 2006; 42: 1441-1447
140. Brunnberg H. RY. Parents' views of sun protection. 2009.
141. Bodekaer Larsen M, Petersen B, Philipsen PA, Young A, Thieden E, Wulf HC. Sun exposure and protection behavior of Danish farm children: parental influence on their children. Photochem Photobiol 2014; 90: 1193-1198
142. Gefeller O, Li J, Uter W, Pfahlberg AB. The impact of parental knowledge and tanning attitudes on sun protection practice for young children in Germany. Int J Environ Res Public Health 2014; 11: 4768-4781
143. Bränström R, Brandberg Y, Holm L, Sjöberg L, Ullen H. Beliefs, knowledge and attitudes as predictors of sunbathing habits and use of sun protection among Swedish adolescents. Eur J Cancer Prev 2001; 10: 337-345

144. Bränström R, Ullen H, Brandberg Y. Attitudes, subjective norms and perception of behavioural control as predictors of sun-related behaviour in Swedish adults. Prev Med 2004; 39: 992-999
145. R. B. Sun habits in Sweden 2007. In: Authority TSRS, editor. 2008.
146. Vagabond travel barometer 2013 2013. Available from: http://www.vagabond.se/artiklar/nyheter/20130528/sa-reser-svenskarna.
147. Sun habits in Sweden 2007. In: Authority TSRS, editor. 2008.
148. Nasti TH, Timares L. MC1R, Eumelanin and Pheomelanin: their role in determining the susceptibility to skin cancer. Photochem Photobiol 2014:
149. Beaumont KA, Wong SS, Ainger SA, Liu YY, Patel MP, Millhauser GL, et al. Melanocortin MC(1) receptor in human genetics and model systems. Eur J Pharmacol 2011; 660: 103-110
150. Mildner M, Jin J, Eckhart L, Kezic S, Gruber F, Barresi C, et al. Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model. J Invest Dermatol 2010; 130: 2286-2294
151. Stiefel C, Schwack W. Photoprotection in changing times - UV filter efficacy and safety, sensitization processes and regulatory aspects. Int J Cosmet Sci 2014:
152. Loden M, Beitner H, Gonzalez H, Edstrom DW, Akerstrom U, Austad J, et al. Sunscreen use: controversies, challenges and regulatory aspects. Br J Dermatol 2011; 165: 255-262
153. van der Pols JC, Williams GM, Pandeya N, Logan V, Green AC. Prolonged prevention of squamous cell carcinoma of the skin by regular sunscreen use. Cancer Epidemiol Biomarkers Prev 2006; 15: 2546-2548
154. Green AC, Williams GM, Logan V, Strutton GM. Reduced melanoma after regular sunscreen use: randomized trial follow-up. J Clin Oncol 2011; 29: 257-263
155. Newman MD, Stotland M, Ellis JI. The safety of nanosized particles in titanium dioxide- and zinc oxide-based sunscreens. J Am Acad Dermatol 2009; 61: 685-692
156. Grether-Beck S, Marini A, Jaenicke T, Krutmann J. Photoprotection of human skin beyond ultraviolet radiation. Photodermatol Photoimmunol Photomed 2014; 30: 167-174
157. Westerdahl J, Ingvar C, Masback A, Olsson H. Sunscreen use and malignant melanoma. Int J Cancer 2000; 87: 145-150
158. Autier P, Dore JF, Negrier S, Lienard D, Panizzon R, Lejeune FJ, et al. Sunscreen use and duration of sun exposure: a double-blind, randomized trial. J Natl Cancer Inst 1999; 91: 1304-1309
159. Kamangar F. Effect modification in epidemiology and medicine. Arch Iran Med 2012; 15: 575-582
160. Smith A, Harrison S, Nowak M, Buettner P, Maclennan R. Changes in the pattern of sun exposure and sun protection in young children from tropical Australia. J Am Acad Dermatol 2013; 68: 774-783
161. Testfakta 2013. Available from: http://www.testfakta.se/tester/f%C3%B6r%C3%A4ldrar-och-barn/s%C3%A5-mycket-f%C3%B6rs%C3%A4mras-skyddet-i-solkl%C3%A4derna.
162. Boldemann C, Blennow M, Dal H, Martensson F, Raustorp A, Yuen K, et al. Impact of preschool environment upon children's physical activity and sun exposure. Prev Med 2006; 42: 301-308

163. Martensson F, Boldemann C, Soderstrom M, Blennow M, Englund JE, Grahn P. Outdoor environmental assessment of attention promoting settings for preschool children. Health Place 2009; 15: 1149-1157
164. Pagels P, Raustorp A, De Leon AP, Martensson F, Kylin M, Boldemann C. A repeated measurement study investigating the impact of school outdoor environment upon physical activity across ages and seasons in Swedish second, fifth and eighth graders. BMC Public Health 2014; 14: 803
165. Randle HW. Suntanning: differences in perceptions throughout history. Mayo Clin Proc 1997; 72: 461-466
166. Howell J. Nobel Laureates in Medicine or Physiology: A Biographical Dictionary Fox DM, Meldrum, M, Rezak I, editors 1990: 181-183
167. Stanton WR, Janda M, Baade PD, Anderson P. Primary prevention of skin cancer: a review of sun protection in Australia and internationally. Health Promot Int 2004; 19: 369-378
168. van der Leest RJ, de Vries E, Bulliard JL, Paoli J, Peris K, Stratigos AJ, et al. The Euromelanoma skin cancer prevention campaign in Europe: characteristics and results of 2009 and 2010. J Eur Acad Dermatol Venereol 2011; 25: 1455-1465
169. Krynitz B, Lindelof B. [The Melanoma Monday saves life and money. Four findings of malignant melanoma among 161 examined persons]. Lakartidningen 2003; 100: 1702-1703
170. Paoli J, Danielsson M, Wennberg AM. Results of the 'Euromelanoma Day' screening campaign in Sweden 2008. J Eur Acad Dermatol Venereol 2009; 23: 1304-1310
171. Maurichi A, Miceli R, Camerini T, Mariani L, Patuzzo R, Ruggeri R, et al. Prediction of survival in patients with thin melanoma: results from a multi-institution study. J Clin Oncol 2014; 32: 2479-2485
172. Boldeman C, Jansson B, Dal H, Ullen H. Sunbed use among Swedish adolescents in the 1990s: a decline with an unchanged relationship to health risk behaviors. Scand J Public Health 2003; 31: 233-237
173. Environmental Health Report 2013 2013. Available from: http://www.imm.ki.se/MHR2013.pdf.
174. English DR, Milne E, Jacoby P, Giles-Corti B, Cross D, Johnston R. The effect of a school-based sun protection intervention on the development of melanocytic nevi in children: 6-year follow-up. Cancer Epidemiol Biomarkers Prev 2005; 14: 977-980
175. Wollina U, Helm C, Bennewitz A, Koch R, Schaff K, Burroni M. Interventional three-year longitudinal study of melanocytic naevus development in pre-school children in Dresden, Saxony. Acta Derm Venereol 2014; 94: 63-66
176. Gallagher RP, Rivers JK, Lee TK, Bajdik CD, McLean DI, Coldman AJ. Broad-spectrum sunscreen use and the development of new nevi in white children: A randomized controlled trial. JAMA 2000; 283: 2955-2960
177. Bergenmar M, Brandberg Y. Sunbathing and sun-protection behaviors and attitudes of young Swedish adults with hereditary risk for malignant melanoma. Cancer Nurs 2001; 24: 341-350
178. Bränström R, Ullen H, Brandberg Y. A randomised population-based intervention to examine the effects of the ultraviolet index on tanning behaviour. Eur J Cancer 2003; 39: 968-974
179. Idorn LW, Datta P, Heydenreich J, Philipsen PA, Wulf HC. A 3-year follow-up of sun behavior in patients with cutaneous malignant melanoma. JAMA Dermatol 2014; 150: 163-168

180. Volkov A, Dobbinson S, Wakefield M, Slevin T. Seven-year trends in sun protection and sunburn among Australian adolescents and adults. Aust N Z J Public Health 2013; 37: 63-69
181. Baade P, Meng X, Youlden D, Aitken J, Youl P. Time trends and latitudinal differences in melanoma thickness distribution in Australia, 1990-2006. Int J Cancer 2012; 130: 170-178
182. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med 2013; 369: 1991-2000
183. Petersen B, Wulf HC, Triguero-Mas M, Philipsen PA, Thieden E, Olsen P, et al. Sun and ski holidays improve vitamin d status, but are associated with high levels of DNA damage. J Invest Dermatol 2014; 134: 2806-2813
184. Bikle DD. Protective actions of vitamin D in UVB induced skin cancer. Photochem Photobiol Sci 2012; 11: 1808-1816
185. Caini S, Boniol M, Tosti G, Magi S, Medri M, Stanganelli I, et al. Vitamin D and melanoma and non-melanoma skin cancer risk and prognosis: A comprehensive review and meta-analysis. Eur J Cancer 2014; 50: 2649-2658
186. Newton-Bishop JA, Beswick S, Randerson-Moor J, Chang YM, Affleck P, Elliott F, et al. Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. J Clin Oncol 2009; 27: 5439-5444
187. Bens G. Sunscreens. Adv Exp Med Biol 2014; 810: 429-463
188. Rettberg P, Horneck G. Intrinsic and extrinsic biomarkers for the assessment of risks from environmental UV radiation. J Epidemiol 1999; 9: S78-83
189. Brandberg Y, Sjoden PO, Rosdahl I. Assessment of sun-related behaviour in individuals with dysplastic naevus syndrome: a comparison between diary recordings and questionnaire responses. Melanoma Res 1997; 7: 347-351
190. Cockburn M, Hamilton A, Mack T. Recall bias in self-reported melanoma risk factors. Am J Epidemiol 2001; 153: 1021-1026
191. Choi BC, Pak AW. A catalog of biases in questionnaires. Prev Chronic Dis 2005; 2: A13
192. Bränström R, Kristjansson S, Ullen H, Brandberg Y. Stability of questionnaire items measuring behaviours, attitudes and stages of change related to sun exposure. Melanoma Res 2002; 12: 513-519
193. Glanz K, Gies P, O'Riordan DL, Elliott T, Nehl E, McCarty F, et al. Validity of self-reported solar UVR exposure compared with objectively measured UVR exposure. Cancer Epidemiol Biomarkers Prev 2010; 19: 3005-3012
194. Liljendahl TS, Blomqvist A, Andersson EM, Barregard L, Segerback D. Urinary levels of thymine dimer as a biomarker of exposure to ultraviolet radiation in humans during outdoor activities in the summer. Mutagenesis 2013; 28: 249-256
195. Kotova N, Hemminki K, Segerback D. Urinary thymidine dimer as a marker of total body burden of UV-inflicted DNA damage in humans. Cancer Epidemiol Biomarkers Prev 2005; 14: 2868-2872
196. Liljendahl TS, Kotova N, Segerback D. Quantification of ultraviolet radiation-induced DNA damage in the urine of Swedish adults and children following exposure to sunlight. Biomarkers 2012; 17: 634-641
197. English DR MR, Rivers J, Kelly J, Armstrong BK, . Epidemiological studies of melanocytic naevi: protocol for identifying and recording naevi. IARC International report, Lyon France 1990; 90/002:

198. English DR, Armstrong BK. Melanocytic nevi in children. II. Observer variation in counting nevi. Am J Epidemiol 1994; 139: 402-407
199. Menon K, Dusza SW, Marghoob AA, Halpern AC, Nehal KS. Classification and prevalence of pigmented lesions in patients with total-body photographs at high risk of developing melanoma. J Cutan Med Surg 2006; 10: 85-91
200. Nehal KS, Oliveria SA, Marghoob AA, Christos PJ, Dusza S, Tromberg JS, et al. Use of and beliefs about baseline photography in the management of patients with pigmented lesions: a survey of dermatology residency programmes in the United States. Melanoma Res 2002; 12: 161-167
201. Terushkin V, Oliveria SA, Marghoob AA, Halpern AC. Use of and beliefs about total body photography and dermatoscopy among US dermatology training programs: an update. J Am Acad Dermatol; 62: 794-803
202. Borve A, Terstappen K, Sandberg C, Paoli J. Mobile teledermoscopy-there's an app for that! Dermatol Pract Concept 2013; 3: 41-48
203. Hirschorn DS, Choudhri AF, Shih G, Kim W. Use of mobile devices for medical imaging. J Am Coll Radiol 2014; 11: 1277-1285
204. Fruhauf J, Hofman-Wellenhof R, Kovarik C, Mulyowa G, Alitwala C, Soyer HP, et al. Mobile teledermatology in sub-Saharan Africa: a useful tool in supporting health workers in low-resource centres. Acta Derm Venereol 2013; 93: 122-123
205. Delaigue S, Morand JJ, Olson D, Wootton R, Bonnardot L. Teledermatology in Low-Resource Settings: The MSF Experience with a Multilingual Tele-Expertise Platform. Front Public Health 2014; 2: 233
206. Ratner D, Thomas CO, Bickers D. The uses of digital photography in dermatology. J Am Acad Dermatol 1999; 41: 749-756
207. Banky JP, Kelly JW, English DR, Yeatman JM, Dowling JP. Incidence of new and changed nevi and melanomas detected using baseline images and dermoscopy in patients at high risk for melanoma. Arch Dermatol 2005; 141: 998-1006
208. Kaliyadan F. Digital photography for patient counseling in dermatology--a study. J Eur Acad Dermatol Venereol 2008; 22: 1356-1358
209. Borve A, Dahlen Gyllencreutz J, Terstappen K, Johansson Backman E, Aldenbratt A, Danielsson M, et al. Smartphone Teledermoscopy Referrals: A Novel Process for Improved Triage of Skin Cancer Patients. Acta Derm Venereol 2014:
210. Chen TS, Goldyne ME, Mathes EF, Frieden IJ, Gilliam AE. Pediatric teledermatology: observations based on 429 consults. J Am Acad Dermatol 2010; 62: 61-66
211. Heffner VA, Lyon VB, Brousseau DC, Holland KE, Yen K. Store-and-forward teledermatology versus in-person visits: a comparison in pediatric teledermatology clinic. J Am Acad Dermatol 2009; 60: 956-961
212. Philp JC, Frieden IJ, Cordoro KM. Pediatric teledermatology consultations: relationship between provided data and diagnosis. Pediatr Dermatol 2013; 30: 561-567
213. Synnerstad I, Nilsson L, Fredrikson M, Rosdahl I. Frequency and distribution pattern of melanocytic naevi in Swedish 8-9-year-old children. Acta Derm Venereol 2004; 84: 271-276
214. Gallus S, Naldi L, Carli P, La Vecchia C, Italian Group for Epidemiologic Research in D. Nevus count on specific anatomic sites as a predictor of total body count: a survey of 3,406 children from Italy. Am J Epidemiol 2007; 166: 472-478
215. Dermicus [Internet]. Available from: http://apegroup.com/project/dermicus.

216. Carmona AB [Internet]. Available from: http://www.carmona.se/ovriga-tjanster/.
217. Augustsson A, Stierner U, Rosdahl I, Suurkula M. Regional distribution of melanocytic naevi in relation to sun exposure, and site-specific counts predicting total number of naevi. Acta Derm Venereol 1992; 72: 123-127
218. Statistics Sweden 2014. Available from: http://www.scb.se/en_/Finding-statistics/Regional-statistics/Regional-divisions/Counties-and-municipalities/.
219. STRÅNG - a mesoscale model for solar radiation. 2009:
220. National Board of Health and Welfare's Cancer Register [Internet]. 2014. Available from: http://www.socialstyrelsen.se/statistics/statisticaldatabase/cancer.
221. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med 1987; 317: 1098
222. Lund CC, Browder NC. The estimation of areas of burns. Surg Gyn Obs 1944; 79:
223. Cohen J. Weighted kappa: nominal scale agreement with provision for scaled disagreement or partial credit. Psychol Bull 1968; 70: 213-220
224. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics 1977; 33: 159-174
225. Ryu E. Model fit evaluation in multilevel structural equation models. Front Psychol 2014; 5: 81
226. Hafner C, Stoehr R, van Oers JM, Zwarthoff EC, Hofstaedter F, Klein C, et al. The absence of BRAF, FGFR3, and PIK3CA mutations differentiates lentigo simplex from melanocytic nevus and solar lentigo. J Invest Dermatol 2009; 129: 2730-2735
227. Aalborg J, Morelli JG, Byers TE, Mokrohisky ST, Crane LA. Effect of hair color and sun sensitivity on nevus counts in white children in Colorado. J Am Acad Dermatol 2010; 63: 430-439
228. Salmela E, Lappalainen T, Liu J, Sistonen P, Andersen PM, Schreiber S, et al. Swedish population substructure revealed by genome-wide single nucleotide polymorphism data. PLoS One 2011; 6: e16747
229. Hassler S, Sjolander P, Gronberg H, Johansson R, Damber L. Cancer in the Sami population of Sweden in relation to lifestyle and genetic factors. Eur J Epidemiol 2008; 23: 273-280
230. Green A, MacLennan R, Youl P, Martin N. Site distribution of cutaneous melanoma in Queensland. Int J Cancer 1993; 53: 232-236
231. Elwood JM, Gallagher RP. Site distribution of malignant melanoma. Can Med Assoc J 1983; 128: 1400-1404
232. Bulliard JL, Cox B, Elwood JM. Comparison of the site distribution of melanoma in New Zealand and Canada. Int J Cancer 1997; 72: 231-235
233. Bulliard JL, De Weck D, Fisch T, Bordoni A, Levi F. Detailed site distribution of melanoma and sunlight exposure: aetiological patterns from a Swiss series. Ann Oncol 2007; 18: 789-794
234. Walter SD, Ashbolt R, Dwyer T, Marrett LD. Do larger people have more naevi? Naevus frequency versus naevus density. Int J Epidemiol 2000; 29: 1025-1030